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(54) Title: NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

(57) Abstract: Novel protein antigens from Group B Streptococcus are described, together with the nucleic acid sequences encoding them. The use of vaccines and screening methods is also described.

Proteins

The present invention relates to proteins derived from *Streptococcus agalactiae*, nucleic acid molecules encoding such proteins, and the use of the proteins as antigens and/or immunogens and in detection/diagnosis. It also relates to a method for the rapid screening of bacterial genomes to isolate and characterise bacterial cell envelope associated or secreted proteins.

The Group B Streptococcus (GBS) *(Streptococcus agalactiae)* is an encapsulated bacterium which emerged in the 1970s as a major pathogen of humans causing sepsis and meningitis in neonates as well as adults. The incidence of early onset neonatal infection during the first 5 days of life varies from 0.7 to 3.7 per 1000 live births and causes mortality in about 20% of cases. Between 25-50% of neonates surviving early onset infections frequently suffer neurological sequalae. Late onset neonatal infections occur from 6 days to three months of age at a rate of about 0.5 - 1.0 per 1000 live births.

There is an established association between the colonisation of the maternal genital tract by GBS at the time of birth and the risk of neonatal sepsis. In humans it has been established that the rectum may act as a reservoir for GBS. Susceptibility in the neonate is correlated with the a low concentration or absence of IgG antibodies to the capsular polysaccharides found on GBS causing human disease. In the USA strains isolated from clinical cases usually belong to capsular serotypes Ia, Ib, II, III although serotype V may be of increasing significance. Type VIII GBS is the major cause of neonatal sepsis in Japan.

A possible means of prevention involves intra or postpartum administration of antibiotics to the mother but there are concerns that this might lead to the emergence

of resistant organisms and in some cases allergic reactions. Vaccination of the adolescent females to induce long lasting maternally derived immunity is one of the most promising approaches to prevent GBS infections in neonates. The capsular polysaccharide antigens of these organisms have attracted most attention as with regard to vaccine development. Studies in healthy adult volunteers have shown that serotype Ia, II and III polysaccharides are non-toxic and immunogenic in approximately 65%, 95% and 70% of non-immune adults respectively. One of the problems with using capsule antigens as vaccines is that the response rates vary according to pre-immunisation status and the polysaccharide antigen and not all vaccinees produce adequate levels of IgG antibody as indicated in vaccination studies with GBS polysaccharides in human volunteers.

Some people do not respond despite repeated stimuli. These properties are due to the T-independent nature of polysaccharide antigens. One strategy to enhance the immunogenicity of these vaccines is to enhance the T cell dependent properties of polysaccharides by conjugating them to a protein. The use of polysaccharide conjugates looks promising but there are still unresolved questions concerning the nature of the carrier protein. A conjugate vaccine against GBS would require at least 4 different conjugates to be prepared adding to the cost of a vaccine.

Approaches to vaccination against GBS infections which rely on the use of capsular polysaccharides have the disadvantage that response rates are likely to vary considerably according to pre-immunisation status and the particular type of polysaccharide antigen used. Results of trials with conjugate vaccines in human volunteers have indicated that response rates may only be around 65% for some of the key capsule antigens (Larsson *et al.*, *Infection and Immunity* **64**:3518-3523 (1996)). It is also not clear whether all individuals responding to the vaccine would have adequate levels of polysaccharide specific IgG which can cross the placenta and

afford immunity to neonates. By conjugating a protein carrier to the polysaccharide antigen it may be possible to convert them to T-cell dependent antigens and enhance their immunogenicity.

Preliminary studies with GBS type III polysaccharide-tetanus toxoid conjugate have been encouraging (Baker *et al.*, *Reviews of Infectious Diseases* **7**:458-467 (1985), Baker *et al.*, *The New England Journal of Medicine* **319**:1180-1185 (1988), Paoletti *et al.*, *Infection and Immunity* **64**:677-679 (1996), Paoletti *et al.*, *Infection and Immunity* **62**:3236-3243 (1994)) but in developed countries the use of tetanus may be disadvantageous since most adults will have been immunised against tetanus within the past five years. Additional boosters with tetanus toxoid may cause adverse reactions (Boyer., *Current Opinions in Pediatrics* **7**:13-18 (1995)). The polysaccharide conjugate vaccines have the disadvantage of being costly to produce and manufacture in comparison with many other kinds of vaccines. There is also the possible risk of problems caused by the cross reactivity between GBS polysaccharides and sialic acid-containing human glycoproteins.

Recent evidence suggests that bacterial surface proteins also may be useful to confer immunity. A protein called Rib which is found on most serotype III strains but rarely on serotypes Ia, Ib or II confers immunity to challenge with Rib expressing GBS in animal models (Stalhammar-Carlemalm *et al.*, *Journal of Experimental Medicine* **177**:1593-1603 (1993)). Another surface protein of interest as a component of a vaccine is the alpha antigen of the C proteins which protected vaccinated mice against lethal infection with strains expressing alpha protein. The amount of this antigen expressed by GBS strains varies markedly, however an alternative to polysaccharides as antigens is the use of protein antigens derived from GBS. Recent evidence suggest that the GBS surface associated proteins Rib and alpha C protein may be used to confer immunity to GBS infections in experimental model systems

(Stalhammar-Carlemalm *et al.*, (1993) [*supra*], Larsson *et al.*, (1996) [*supra*]). However these two proteins are not conserved in all serotypes of GBS which cause disease in humans. Assuming that these antigens would be immunogenic and elicit protective level responses in humans they would not confer protection against all infections caused by GBS as 10% of infectious Group B streptococci do not express Rib or C protein alpha.

This invention seeks to overcome the problem of vaccination against GBS by using a novel screening method specifically designed to identify those Group B Streptococcus genes encoding bacterial cell surface associated or secreted proteins. The proteins expressed by these genes may be immunogenic, and therefore may be useful in the prevention and treatment of Group B Streptococcus infection. For the purposes of this application, the term immunogenic means that these proteins will elicit a protective immune response within a subject. Using this novel screening method a number of genes encoding novel Group B Streptococcus proteins have been identified.

Thus in a first aspect, the present invention provides a Group B Streptococcus protein, polypeptide or peptide having a sequence selected from those shown in figure 1, or fragments or derivatives thereof.

It will be apparent to the skilled person that proteins and polypeptides included within this group may be cell surface receptors, adhesion molecules, transport proteins, membrane structural proteins, and/or signalling molecules.

Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation

start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the protein's function.

Thus, the present invention includes derivatives or variants of the proteins, polypeptides, and peptides of the present invention which show at least 50% identity to the proteins, polypeptides and peptides described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still it is above 80%, 90% or even 95%.

The term identity can be used to describe the similarity between two polypeptide sequences. A software package well known in the art for carrying out this procedure is the CLUSTAL program. It compares the amino acid sequences of two polypeptides and finds the optimal alignment by inserting spaces in either sequence as appropriate. The amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment can also be calculated using a software package such as BLASTx. This program aligns the largest stretch of similar sequence and assigns a value to the fit. For any one pattern comparison several regions of similarity may be found, each having a different score. One skilled in the art will appreciate that two polypeptides of different lengths may be compared over the entire length of the longer fragment. Alternatively small regions may be compared. Normally sequences of the same length are compared for a useful comparison to be made.

Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to

design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

Typically primers will be at least five nucleotides long and will generally be at least ten nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases primers of at least thirty or at least thirty-five nucleotides in length may be used.

As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to provide a longer sequence.

Thus in a further aspect, the present invention provides, a nucleic acid molecule comprising or consisting of a sequence which is:

(i) any of the DNA sequences set out in figure 1 herein or their RNA equivalents;

(ii) a sequence which is complementary to any of the sequences of (i);

(iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);

(iv) a sequence which is shows substantial identity with any of those of (i), (ii) and (iii); or

(v) a sequence which codes for a derivative or fragment of a nucleic acid molecule shown in figure 1.

The term identity can also be used to describe the similarity between two individual DNA sequences. The 'bestfit' program (Smith and Waterman, *Advances in applied Mathematics*, 482-489 (1981)) is one example of a type of computer software used to find the best segment of similarity between two nucleic acid sequences, whilst the GAP program enables sequences to be aligned along their whole length and finds the optimal alignment by inserting spaces in either sequence as appropriate.

The present invention includes nucleic acid sequences which show at least 50% identity to the nucleic acid sequences described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still it is above 80%, 90% or even 95%.

The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may be in isolated, recombinant or chemically synthetic form.

DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2nd Edition*, Cold Spring Harbour Laboratory Press (1989). Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

Normally the DNA construct will be inserted into a vector which may be any suitable vector, including plasmid, virus, bacteriophage, transposon, minichromosome, liposome or mechanical carrier. The expression vectors of the invention are DNA constructs suitable for expressing DNA which encodes the desired protein product which may include: (a) a regulatory element (e.g. a promoter, operator, activator, repressor and/or enhancer), (b) a structural or coding sequence which is transcribed into mRNA and (c) appropriate transcription, translation, initiation and termination sequences. The vector may further comprise a selectable marker, for example antibiotic resistance, which facilitates the selection and/or identification of cells containing the vector.

Expression of the protein is achieved by the transformation or transfection of the vector into a host cell which may be of eukaryotic or prokaryotic origin. For the production of recombinant protein, expression may be inducible expression or expression only in certain types of cells or both inducible and cell-specific. Particularly preferred among inducible vectors are vectors that can be induced for expression by environmental factors that are easy to manipulate, such as temperature and nutrient additives. A variety of suitable vectors, including constitutive and inducible expression vectors for use in prokaryotic and eukaryotic hosts, are well known and employed routinely by those skilled in the art.

A great variety of expression vectors can be used to express the Group B Streptococcus protein(s) of the invention. Such vectors include, among others, chromosomal, episomal and virus-derived vectors, for example, vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids, all may be used in accordance with the invention. Generally, any vector suitable to maintain, propagate or express nucleic acid to express a polypeptide in a host may be used for expression in this regard. Such vectors thus form yet a further aspect of the invention.

The appropriate DNA sequence may be inserted into the vector by any of a variety of well-known and routine techniques.

The nucleic acid sequence in the expression vector is operatively linked to appropriate expression control sequence(s) including, for instance, a promoter to

direct mRNA transcription. Representatives of such promoters include, but are not limited to, the phage lambda PL promoter, the T3 and T7 promoters, the *E.coli* lac, trp, tac, and λP_L promoters, the microbial eukaryote GAL, glucoamylase and cellobiohydrolase promoters and the mammalian metallothionein (mouse) and heat-shock (human) promoters.

In general, expression vectors will contain sites for transcription initiation and termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of mature transcripts expressed by the constructs will generally include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

Representative examples of appropriate hosts for recombinant expression of the Group B Streptococcus protein(s) of the invention include bacterial cells, such as *streptococci*, *staphylococci*, *E.coli*, *streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa and Bowes melanoma cells; and plant cells. Such host cells form yet a further aspect of the present invention.

Microbial cells employed in the expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agent, such methods which are known to those skilled in the art.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose,

chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

The Group B Streptococcus proteins described herein can additionally be used as target antigens to raise antibodies, or to generate affibodies. These can be used to detect Group B Streptococcus.

Thus in a further aspect the present invention provides, an antibody, affibody, or a derivative thereof which binds to any one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as described herein.

Antibodies within the scope of the present invention may be monoclonal or polyclonal. Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when a protein as described herein, or a homologue, derivative or fragment thereof, is injected into the animal. If desired, an adjuvant may be administered together with the protein. Well-known adjuvants include Freund's adjuvant (complete and incomplete) and aluminium hydroxide. The antibodies can then be purified by virtue of their binding to a protein as described herein and by many other means well-known to those skilled in the art.

Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. Thus the well-known Kohler & Milstein technique (*Nature* **256** (1975)) or subsequent variations upon this technique can be used.

Techniques for producing monoclonal and polyclonal antibodies that bind to a particular polypeptide/protein are now well developed in the art. They are discussed in standard immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to proteins etc as described herein. Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* ., *Tibtech* **12** 372-379 (September 1994).

Antibody fragments include, for example, Fab, $F(ab')_2$ and Fv fragments. Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V_h and V_l regions, which contributes to the stability of the molecule. Other synthetic constructs that can be used include CDR peptides. These are synthetic peptides comprising antigen-binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings that mimic the structure of a CDR loop and that include antigen-interactive side chains.

Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Ways of producing chimaeric antibodies are discussed for example by Morrison *et al* in *PNAS*, **81,** 6851-6855 (1984) and by Takeda *et al* in *Nature*. **314,** 452-454 (1985).

Synthetic constructs also include molecules comprising an additional moiety that provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label). Alternatively, it may be a pharmaceutically active agent.

Affibodies are proteins which are found to bind to target proteins with a low dissociation constant. They are selected from phage display libraries expressing a segment of the target protein of interest (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA, Department of Biochemistry and Biotechology, Royal Institute of Technology (KTH), Stockholm, Sweden).

In a further aspect the invention provides an immunogenic composition comprising one or more proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleotide sequences described herein. The immunogenic composition may include nucleic acid sequences ID-65 and/or ID-66 as described herein. Alternatively, the immunogenic composition may comprise proteins/polypeptides including ID-65, ID-83, ID-89, ID-93 and/or ID-96 as described herein, or fragments or derivatives thereof. A composition of this sort may be useful in the treatment or prevention of Group B Streptococcus infection in subject. In a preferred aspect of the invention the immunogenic composition is a vaccine.

In other aspects the invention provides:

i) Use of an immunogenic composition as described herein in the preparation of a medicament for the treatment or prophylaxis of Group B Streptococcus infection. Preferably the medicament is a vaccine.

ii) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.

iii) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.

iv) A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.

v) A kit for the detection of Group B Streptococcus comprising at least one antibody, affibody, or derivatives thereof, described herein.

vi) A kit for the detection of Group B Streptococcus comprising at least one Group B Streptococcus protein, polypeptide, peptide, fragment or derivative thereof, as described herein.

vii) A kit for the detection of Group B Streptococcus comprising at least one nucleic acid of the invention.

As described previously, the novel proteins described herein are identified and isolated using a screening method which specifically identifies those Group B Streptococcus genes encoding bacterial cell envelope associated or secreted proteins.

Given that the inventors have identified a group of important proteins, such proteins are potential targets for anti-microbial therapy. It is necessary, however, to

determine whether each individual protein is essential for the organism's viability. Thus, the present invention also provides a method of determining whether a protein or polypeptide as described herein represents a potential anti-microbial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

A suitable method for inactivating the protein is to effect selected gene knockouts, ie prevent expression of the protein and determine whether this results in a lethal change. Suitable methods for carrying out such gene knockouts are described in Li *et al , P.N.A.S.,* **94**:13251-13256 (1997) and Kolkman *et al., Journal of Biological Chemistry* **272**: 19502-19508 (1997); Kolkman *et al., Journal of Bacteriology* **178**: 3736-3741 (1996).

In a final aspect the present invention provides the use of an agent capable of antagonising, inhibiting or otherwise interfering with the function or expression of a protein or polypeptide of the invention in the manufacture of a medicament for use in the treatment or prophylaxis of Group B Streptococcus infection.

The invention will now be described by means of the following examples which should not in any way be construed as limiting. The examples refer to the figures in which:

Fig 1: (A) Shows a number of full length nucleotide sequences encoding antigenic Group B Streptococcus proteins and the corresponding amino acid sequences.

Fig 2: Shows the results of vaccine trials using the proteins ID-65 and ID-66;

Fig 3: Shows a number of oligonucleotide primers used in the screening process

nucS1 primer designed to amplify a mature form of the nuc A gene

nucS2- primer designed to amplify a mature form of the nuc A gene.

nucS3 primer designed to amplify a mature form of the nuc A gene

nucR primer designed to amplify a mature form of the nuc A gene

nucseq primer designed to sequence DNA cloned into the pTREP-Nuc vector

pTREPF nucleic acid sequence containing recognition site for ECORV. Used for cloning fragments into pTREX7.

pTREPR nucleic acid sequence containing recognition site for BAMH1. Used for cloning fragments into pTREX7.

PUCF forward sequencing primer, enables direct sequencing of cloned DNA fragments.

VR example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

V1 example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

V2 example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

Fig 4: (i) Schematic presentation of the nucleotide sequence of the unique gene cloning site immediately upstream of the mature *nuc* gene in pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3. Each of the pTREP-*nuc* vectors contain an EcoRV (a SmaI site in pTREP1-*nuc*2) cleavage site which allows cloning of genomic DNA fragments in 3 different frames with respect to the mature *nuc* gene.

(ii) A physical and genetic summary map of the pTREP1-*nuc* vectors. The expression cassette incorporating *nuc*, the macrolides, lincosamides and

streptogramin B (MLS) resistance determinant, and the replicon (rep) *Ori*-pAMβ1 are depicted (not drawn to scale).

(iii) Schematic presentation of the expression cassette showing the various sequence elements involved in gene expression and location of unique restriction endonuclease sites (not drawn to scale).

Fig 5: SDS-PAGE analysis of a purified preparation of the His-tagged ID-65 and ID-83 protein antigens (predicted molecular weights of 57,144 and 25,000 daltons respectively) on a 12% polyacrylamide gel. Lanes: MW, molecular weight standards; 1, His-tagged ID-65 protein; 2, His-tagged ID-83 protein

Fig 6: SDS PAGE analysis of a purified preparation of the His-tagged ID-93 protein antigen (predicted molecular weight = 28,000 daltons) on a 12% polyacrylamide gel.

Lanes: MW, molecular weight standards; 1, His-tagged ID-93 protein.

Fig 7: SDS PAGE analysis of a purified preparation of the His-tagged ID-89 and ID-96 protein antigens (predicted molecular weights of 35,000 and 31,000 daltons respectively) on a 12% polyacrylamide gel.

Lanes: MW, molecular weight standards; 1, His-tagged ID-89 protein; 2, His-tagged ID-96 protein.

Fig 8: IgG Titres against the ID-65 and ID-83 proteins

1 = ID-65 + Alum Group – Bleed at 5 weeks

2 = PBS + Alum Control Group – Bleed at 5 weeks

(For groups 1 and 2, ELISAs were performed on purified ID-65 protein)

3 = ID-83 + Alum Group – Bleed at 5 weeks

4 = PBS+ Alum Control Group – Bleed at 5 weeks

(For groups 3 and 4, ELISAs were performed on purified ID-83 protein)

Fig 9: Shows the results of vaccine trials using the protein ID-93.

Fig 10: IgG titres against the ID-93 protein.

1 = ID-93+Alum Group – Bleed at 3 weeks

2 = ID-93+Alum Group – Bleed at 6 weeks

3 = PBS+Alum Control Group – Bleed at 3 weeks

4 = PBS+Alum Control Group – Bleed at 6 weeks

Fig 11: IgG titres against the ID-89 and ID-96 proteins

1 = ID-89+TitreMax Gold Group – Bleed at 3 weeks

2 = ID-89+ TitreMax Gold – Bleed at 6 weeks

3 = PBS+ TitreMax Gold Control Group – Bleed at 3 weeks

4 = PBS+ TitreMax Gold Control Group – Bleed at 6 weeks

5 = ID-96+ TitreMax Gold Group – Bleed at 3 weeks

6 = ID-96+ TitreMax Gold Group – Bleed at 6 weeks

7 = PBS+ TitreMax Gold Control Group – Bleed at 3 weeks

8 = PBS+ TitreMax Gold Control Group – Bleed at 6 weeks

For Groups 1-4, ELISAs were performed on purified ID-89 protein.

For Groups 5-6, ELISAs were performed on purified ID-96 protein.

Fig 12: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 7 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the

digoxigenin-labelled *rib* gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Fig 13: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-65 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Fig 14: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with Hin DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-89 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Fig 15: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled ID-93 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Fig 16: Southern blot analysis of genomic DNA. Genomic DNA from each of the strains listed in Table 6 was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the

digoxigenin-labelled ID-96 gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Example 1

Gene/partial gene sequences putatively encoding exported proteins in *S. agalactiae* have been identified, unless stated otherwise, using the nuclease screening system described herein *vis*, the LEEP (Lactococcus Expression of Exported Proteins) system. These have been further analysed to remove artefacts. The nucleotide sequences of genes identified using the screening system have been characterised using a number of parameters described below.

1. All putative surface proteins are analysed for leader/signal peptide sequences. Bacterial signal peptide sequences share a common design. They are characterised by a short positively charged N-terminus (N region) immediately preceding a stretch of hydrophobic residues (central portion-h region) followed by a more polar C-terminal portion which contains the cleavage site (c-region). Computer software is used to perform hydropathy profiling of putative proteins (Marcks, *Nuc. Acid. Res.*, **16**:1829-1836 (1988)) which is used to identify the distinctive hydrophobic portion (h-region) typical of leader peptide sequences. In addition, the presence/absence of a potential ribosomal binding site (Shine-Dalgarno sequence required for translation) is also noted.

2. All putative surface protein sequences are used to search the OWL sequence database which includes a translation of the GENBANK and SWISSPROT database.. This allows identification of similar sequences which may have been previously characterised not only at the sequence level but at a functional level. It

may also provide information indicating that these proteins are indeed surface related and not artefacts.

3. Putative *S. agalactiae* surface proteins are also assessed for their novelty. Some of the identified proteins may or may not possess a typical leader peptide sequence and may not show homology with any DNA/protein sequences in the database. Indeed these proteins may indicate the primary advantage of our screening method, i.e. isolating atypical surface-related proteins, which would have been missed in all previously described screening protocols.

The construction of three reporter vectors and their use in *L. lactis* to identify and isolate genomic DNA fragments from pathogenic bacteria encoding secreted or surface associated proteins is now described.

Construction of the pTREP1-*nuc* series of reporter vectors

(a) Construction of expression plasmid pTREP1

The pTREP1 plasmid is a high-copy number (40-80 per cell) theta-replicating gram positive plasmid, which is a derivative of the pTREX plasmid which is itself a derivative of the previously published pIL253 plasmid. pIL253 incorporates the broad Gram-positive host range replicon of pAMβ1 (Simon and Chopin, *Biochemie* **70**: 559-566 (1988))*L lactis* sex-factor. pIL253 also lacks the *tra* function which is necessary for transfer or efficient mobilisation by conjugative parent plasmids exemplified by pIL501. The Enterococcal pAMβ1 replicon has previously been transferred to various species including *Streptococcus, Lactobacillus* and *Bacillus* species as well as *Clostridium acetobutylicum*, (LeBlanc *et al.*, *Proceedings of the National Academy of Science USA* **75:**3484-3487 (1978)) indicating the potential broad host range utility. The pTREP1 plasmid represents a constitutive transcription vector.

The pTREX vector was constructed as follows. An artificial DNA fragment containing a putative RNA stabilising sequence, a translation initiation region (TIR), a multiple cloning site for insertion of the target genes and a transcription terminator was created by annealing 2 complementary oligonucleotides and extending with Tfl DNA polymerase. The sense and anti-sense oligonucleotides contained the recognition sites for NheI and BamHI at their 5' ends respectively to facilitate cloning. This fragment was cloned between the XbaI and BamHI sites in pUC19NT7, a derivative of pUC19 which contains the T7 expression cassette from pLET1 (Wells *et al., J. Appl. Bacteriol.* **74:**629-636 (1993)) cloned between the EcoRI and HindIII sites. The resulting construct was designated pUCLEX. The complete expression cassette of pUCLEX was then removed by cutting with HindIII and blunting followed by cutting with EcoRI before cloning into EcoRI and SacI (blunted) sites of pIL253 to generate the vector pTREX (Wells and Schofield, *In* Current advances in metabolism, genetics and applications-NATO ASI Series. **H 98:**37-62. (1996)). The putative RNA stabilising sequence and TIR are derived from the *Escherichia coli* T7 bacteriophage sequence and modified at one nucleotide position to enhance the complementarity of the Shine Dalgarno (SD) motif to the ribosomal 16s RNA of *Lactococcus lactis* (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.).

A *Lactococcus lactis* MG1363 chromosomal DNA fragment exhibiting promoter activity which was subsequently designated P7 was cloned between the EcoRI and BglII sites present in the expression cassette, creating pTREX7. This active promoter region had been previously isolated using the promoter probe vector pSB292 (Waterfield *et al.*, *Gene* **165:**9-15 (1995)). The promoter fragment was amplified by PCR using the Vent DNA polymerase according to the manufacturer.

The pTREP1 vector was then constructed as follows. An artificial DNA fragment which included a transcription terminator, the forward pUC sequencing primer, a promoter multiple cloning site region and a universal translation stop sequence was created by annealing two overlapping partially complementary synthetic oligonucleotides together and extending with sequenase according to manufacturers instructions. The sense and anti-sense ($pTREP_F$ and $pTREP_R$) oligonucleotides contained the recognition sites for EcoRV and BamHI at their 5' ends respectively to facilitate cloning into pTREX7. The transcription terminator was that of the *Bacillus penicillinase* gene, which has been shown to be effective in Lactococcus (Jos *et al.*, *Applied and Environmental Microbiology* **50:**540-542 (1985)). This was considered necessary as expression of target genes in the pTREX vectors was observed to be leaky and is thought to be the result of cryptic promoter activity in the origin region (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.). The forward pUC primer sequencing was included to enable direct sequencing of cloned DNA fragments. The translation stop sequence which encodes a stop codon in 3 different frames was included to prevent translational fusions between vector genes and cloned DNA fragments. The pTREX7 vector was first digested with EcoRI and blunted using the 5' - 3' polymerase activity of T4 DNA polymerase (NEB) according to manufacturer's instructions. The EcoRI digested and blunt ended pTREX7 vector was then digested with Bgl II thus removing the P7 promoter. The artificial DNA fragment derived from the annealed synthetic oligonucleotides was then digested with EcoRV and Bam HI and cloned into the EcoRI(blunted)-Bgl II digested pTREX7 vector to generate pTREP. A *Lactococcus lactis* MG1363 chromosomal promoter designated P1 was then cloned between the EcoRI and BglII sites present in the pTREP expression cassette forming pTREP1. This promoter was also isolated using the promoter probe vector pSB292 and characterised by Waterfield *et al.*, (1995) [*supra*]. The P1 promoter fragment was originally amplified by PCR using vent DNA polymerase according to manufacturers

instructions and cloned into the pTREX as an EcoRI-BglII DNA fragment. The EcoRI-BglII P1 promoter containing fragment was removed from pTREX1 by restriction enzyme digestion and used for cloning into pTREP (Schofield *et al.* pers. coms. University of Cambridge, Dept. Pathology.).

(b) PCR amplification of the *S. aureus nuc* gene.

The nucleotide sequence of the *S. aureus nuc* gene (EMBL database accession number V01281) was used to design synthetic oligonucleotide primers for PCR amplification. The primers were designed to amplify the mature form of the *nuc* gene designated *nuc*A which is generated by proteolytic cleavage of the N-terminal 19 to 21 amino acids of the secreted propeptide designated Snase B (Shortle, 1983 [*supra*]). Three sense primers (*nuc*S1, *nuc*S2 and *nuc*S3, shown in figure 3) were designed, each one having a blunt-ended restriction endonuclease cleavage site for EcoRV or SmaI in a different reading frame with respect to the *nuc* gene. Additionally BglII and BamHI were incorporated at the 5' ends of the sense and anti-sense primers respectively to facilitate cloning into BamHI and BglII cut pTREP1. The sequences of all the primers are given in figure 3. Three *nuc* gene DNA fragments encoding the mature form of the nuclease gene (*Nuc*A) were amplified by PCR using each of the sense primers combined with the anti-sense primer. The *nuc* gene fragments were amplified by PCR using *S. aureus* genomic DNA template, Vent DNA Polymerase (NEB) and the conditions recommended by the manufacturer. An initial denaturation step at 93°C for 2 min was followed by 30 cycles of denaturation at 93°C for 45 sec, annealing at 50°C for 45 seconds, and extension at 73°C for 1 minute and then a final 5 min extension step at 73°C. The PCR amplified products were purified using a Wizard clean up column (Promega) to remove unincorporated nucleotides and primers.

(c) Construction of the pTREP1-*nuc* vectors

The purified *nuc* gene fragments described in section b were digested with Bgl II and BamHI using standard conditions and ligated to BamHI and BglII cut and dephosphorylated pTREP1 to generate the pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3 series of reporter vectors. These vectors are described in figure 4. General molecular biology techniques were carried out using the reagents and buffers supplied by the manufacturer or using standard techniques (Sambrook and Maniatis, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbour (1989)). In each of the pTREP1-*nuc* vectors the expression cassette comprises a transcription terminator, lactococcal promoter P1, unique cloning sites (Bgl II, EcoRV or SmaI) followed by the mature form of the *nuc* gene and a second transcription terminator. Note that the sequences required for translation and secretion of the *nuc* gene were deliberately excluded in this construction. Such elements can only be provided by appropriately digested foreign DNA fragments (representing the target bacterium) which can be cloned into the unique restriction sites present immediately upstream of the *nuc* gene.

(d) Screening for secreted proteins in Group B Streptococcus.

Genomic DNA isolated from Group B Streptococcus *(S. agalactiae)* was digested with the restriction enzyme Tru9I. This enzyme which recognises the sequence 5'-TTAA -3' was used because it cuts A/T rich genomes efficiently and can generate random genomic DNA fragments within the preferred size range (usually averaging 0.5 - 1.0 kb). This size range was preferred because there is an increased probability that the P1 promoter can be utilised to transcribe a novel gene sequence. However, the P1 promoter may not be necessary in all cases as it is possible that many Streptococcal promoters are recognised in *L. lactis*. DNA fragments of different size ranges were purified from partial Tru9I digests of *S. agalactiae* genomic DNA. As

the Tru 9I restriction enzyme generates staggered ends the DNA fragments had to be made blunt ended before ligation to the EcoRV or SmaI cut pTREP1-*nuc* vectors. This was achieved by the partial fill-in enzyme reaction using the 5'-3' polymerase activity of Klenow enzyme. Briefly Tru9I digested DNA was dissolved in a solution (usually between 10-20 μl in total) supplemented with T4 DNA ligase buffer (New England Biolabs; NEB) (1X) and 33 μM of each of the required dNTPs, in this case dATP and dTTP. Klenow enzyme was added (1 unit Klenow enzyme (NEB) per μg of DNA) and the reaction incubated at 25°C for 15 minutes. The reaction was stopped by incubating the mix at 75°C for 20 minutes. EcoRV or SmaI digested pTREP-*nuc* plasmid DNA was then added (usually between 200-400 ng). The mix was then supplemented with 400 units of T4 DNA ligase (NEB) and T4 DNA ligase buffer (1X) and incubated overnight at 16°C. The ligation mix was precipitated directly in 100% Ethanol and 1/10 volume of 3M sodium acetate (pH 5.2) and used to transform *L. lactis* MG1363 (Gasson, *J. Bacteriol.* **154:**1-9 (1983)). Alternatively, the gene cloning site of the pTREP-*nuc* vectors also contains a BglII site which can be used to clone for example Sau3AI digested genomic DNA fragments.

L. lactis transformant colonies were grown on brain heart infusion agar and nuclease secreting (*Nuc*$^+$) clones were detected by a toluidine blue-DNA-agar overlay (0.05 M Tris pH 9.0, 10 g of agar per litre, 10 g of NaCl per liter, 0.1 mM CaCl2, 0.03% wt/vol. salmon sperm DNA and 90 mg of Toluidine blue O dye) essentially as described by Shortle, 1983 [*supra*], and Le Loir *et al.*, 1994 [*supra*]). The plates were then incubated at 37°C for up to 2 hours. Nuclease secreting clones develop an easily identifiable pink halo. Plasmid DNA was isolated from *Nuc*$^+$ recombinant *L. lactis* clones and DNA inserts were sequenced on one strand using the *Nuc*Seq sequencing primer described in figure 3, which sequences directly through the DNA insert.

Example 2

Preparation of a *S. agalactiae* standard inoculum

Strain validation

S. agalactiae serotype III (strain 97/0099) is a recent clinical isolate derived from the cerebral spinal fluid of a new born baby suffering from meningitis. This haemolytic strain of Group B Streptococcus was epidemiologically tested and validated at the Respiratory and Systemic Infection Laboratory, PHLS Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT. The strain was subcultured only twice prior to its arrival in the laboratory. Upon its arrival on an agar slope, a sweep of 4-5 colonies was immediately used to inoculate a Todd Hewitt/5% horse blood broth which was incubated overnight statically at 37°C. 0.5 ml aliquots of this overnight culture were then used to make 20% glycerol stocks of the bacterium for long-term storage at -70°C. Glycerol stocks were streaked on Todd Hewitt/5% horse blood agar plates to confirm viability.

***In vivo* passaging of Group B Streptoccocus**

A frozen culture (described under strain validation) of *S. agalactiae* serotype III (strain 97/0099) was streaked to single colonies on Todd-Hewitt/5% blood agar plates, which were incubated overnight at 37°C. A sweep of 4-5 colonies was used to inoculate a Todd Hewitt/5% horse blood broth, which was again incubated overnight. A 0.5 ml aliquot from this overnight culture was used to inoculate a 50 ml Todd Hewitt broth (1:100 dilution) which was incubated at 37°C. 10-fold serial dilutions of the overnight culture were made (since virulence of this strain was unknown) and each was passaged intra-peritoneally (IP) in CBA/ca mice in duplicate. Viable counts were performed on the various inocula used in the passage. Groups of mice were challenged with various concentrations of the pathogen ranging from 10^8 to 10^4 colony forming units (cfu). Mice that developed symptoms were terminally anaesthetized and cardiac punctures were performed (Only mice that had

been challenged with the highest doses, i.e. 1 X 10^8 cfu, developed symptoms). The retrieved unclotted blood was used to inoculate directly a 50ml serum broth (Todd Hewitt/20% inactivated foetal calf serum). The culture was constantly monitored and allowed to grow to late logarithmic phase. The presence of blood in the medium interfered with OD_{600nm} readings as it was being increasingly lysed with increasing growth of the bacterium, hence the requirement to constantly monitor the culture. Upon reaching late logarithmic phase/early stationary phase, the culture was transferred to a fresh 50 ml tube in order to exclude dead bacterial cells and remaining blood cells which would have sedimented at the bottom of the tube. 0.5 ml aliquots were then transferred to sterile cryovials, frozen in liquid nitrogen and stored at -70°C. A viable count was carried out on a single standard inoculum aliquot in order to determine bacterial numbers. This was determined to be approximately 5 X10^8 cfu per ml.

Intra-peritoneal Challenge and virulence testing of Group B Streptococcus standard inoculum

To determine if the standard inoculum was suitably virulent for use in a vaccine trial, challenges were carried out using a dose range. Frozen standard inoculum strain aliquots were allowed to thaw at room temperature. From viable count data the number of cfu per ml was already known for the standard inoculum. Initially, serial dilutions of the standard inoculum were made in Todd Hewitt broth and mice were challenged intra-peritoneally with doses ranging from 1 X 10^8 to 1 X 10^4 cfu in a 500 μl volume of Todd Hewitt broth. The survival times of mouse groups injected with different doses of the bacterium were compared. The standard inoculum was determined to be suitably virulent and a dose of 1 X 10^6 cfu was considered close to optimal for further use in vaccine trials. Further optimisation was carried out by comparing mice challenged with doses ranging between 5 X 10^5 and 5 X 10^6 cfu. The optimal dose was estimated to be approximately 2.5 X10^6 cfu. This represented

a 100% lethal dose and was repeatedly consistent with end-points as determined by survival times being clustered within a narrow time-range. Throughout all these experiments, challenged mice were constantly monitored to clarify symptoms, stages of symptom development as well as calculating survival times.

Screening Group B Streptococcal LEEP derived genes in DNA vaccination experiments.

pcDNA3.1+ as a DNA vaccine vector

The commercially available pcDNA3.1+ plasmid (Invitrogen), referred to as pcDNA3.1 henceforth, was used as a vector in all DNA immunisation experiments involving gene targets derived using the LEEP system unless stated otherwise. pcDNA 3.1 is designed for high-level stable and transient expression in mammalian cells and has been used widely and successfully as a host vector to test candidate genes from a variety of pathogens in DNA vaccination experiments (Zhang *et al.*, *Infection and Immunity* **176:** 1035-40 (1997); Kurar and Splitter, *Vaccine* **15:** 1851-57 (1997); Anderson *et al.*, *Infection and Immunity* **64:** 3168-3173 (1996)).

The vector possesses a multiple cloning site which facilitates the cloning of multiple gene targets downstream of the human cytomegalovirus (CMV) immediate-early promoter/enhancer which permits efficient, high-level expression of the target gene in a wide variety of mammalian cells and cell types including both muscle and immune cells. This is important for optimal immune response as it remains unknown as to which cells types are most important in generating a protective response *in vivo*. The plasmid also contains the ColE1 origin of replication which allows convenient high-copy number replication and growth in *E. coli* and the ampicillin resistance gene (B- lactamase) for selection in *E. coli*. In addition pcDNA 3.1

possesses a T7 promoter/priming site upstream of the MCS which allows for *in vitro* transcription of a cloned gene in the sense orientation.

Preparation of DNA vaccines

Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system unless stated otherwise. Each gene was examined thoroughly, and where possible, primers were designed such that they targeted that portion of the gene believed to encode only the mature portion of the protein (APPENDIX I); the intention being to express those sequences that encode only the mature portion of a target gene protein to would facilitate its correct folding when expressed in mammalian cells. For example, in the majority of cases primers were designed such that putative N-terminal signal peptide sequences would not be included in the final amplification product to be cloned into the pcDNA3.1 expression vector. The signal peptide directs the polypeptide precursor to the cell membrane via the protein export pathway where it is normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein). Hence the signal peptide does not make up any part of the mature protein whether it be displayed on the bacterium's surface or secreted. Where an N-terminal leader peptide sequence was not immediately obvious, primers were designed to target the whole of the gene sequence for cloning and ultimately, expression in pcDNA3.1.

All forward and reverse oligonucleotide primers incorporated appropriate restriction enzyme sites to facilitate cloning into the pcDNA3.1 MCS region. All forward primers were also designed to include the conserved Kozak nucleotide sequence 5'-gccacc-3' immediately upstream of an 'atg' translation initiation codon in frame with the target gene insert. The Kozak sequence facilitates the recognition of initiator sequences by eukaryotic ribosomes. Typically, a forward primer incorporating a BamH1 restriction enzyme site the primer would begin with the sequence 5'-

cgggatccgccaccatg-3', followed by a sequence homologous to the 5' end of that part of a gene being amplified. All reverse primers incorporated a Not I restriction enzyme site sequence 5' -ttgcggccgc-3'. All gene-specific forward and reverse primers were designed with compatible melting temperatures to facilitate their amplification.

All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template using Vent DNA polymerase (NEB) or rT*th DNA* polymerase (*PE* Applied Biosystems) using conditions recommended by the manufacturer. A typical amplification reaction involved an initial denaturation step at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). This was followed by a final extension period at 72°C for 10 minutes. All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). The purified amplification gene DNA fragments were digested with the appropriate restriction enzymes and cloned into the pcDNA3.1 plasmid vector using *E. coli* as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping and by DNA sequencing. Recombinant plasmid DNA was isolated on a large scale (>1.5 mg) using Plasmid Mega Kits (Qiagen).

DNA vaccination trials

DNA vaccine trials in mice were accomplished by the administration of DNA to 6 week old CBA/ca mice (Harlan, UK). Mice to be vaccinated were divided into groups of six and each group was immunised with recombinant pcDNA3.1 plasmid DNA containing a specific target-gene sequence derived using the LEEP system unless stated otherwise. A total of 100 μg of DNA in Dulbecco's PBS (Sigma) was

injected intramuscularly into the tibialis anterior muscle of both hind legs. Four weeks later this procedure was repeated using the same amount of DNA. For comparison, control mice groups were included in all vaccine trials. These control groups were either not DNA-vaccinated or were immunised with non-recombinant pcDNA3.1 plasmid DNA only, using the same time course described above. Four weeks after the second immunisation, all mice groups were challenged intra-peritoneally with a lethal dose of *S. agalactiae* serotype III (strain 97/0099). The actual number of bacteria administered was determined by plating serial dilutions of the inoculum on Todd-Hewitt/5% blood agar plates. All mice were killed 3 or 4 days after infection. During the infection process, challenged mice were monitored for the development of symptoms associated with the onset of *S. agalactiae* induced-disease. Typical symptoms in an appropriate order included piloerection, an increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death. The results of this trial are shown in Table 1and presented graphically in Figure 2.

Interpretation of Results

A positive result was taken as any DNA sequence that was cloned and used in challenge experiments as described above and gave protection against that challenge. DNA sequences were determined to be protective;

-if that DNA sequence gave statistically significant protection to mice as compared to control mice (to a 95% confidence level ($p > 0.05$) as determined using the Mann-Whitney U test .

-if that DNA sequence was marginal or non-signficant using Mann-Whitney but showed some protective features. For example, one or more outlying mice may survive for significantly longer time periods when compared with control mice. Alternatively, the time to first death may also be prolonged when compared to counterpart mice in control groups. It is acceptable to allow marginal or non-significant results to be considered as potential positives when it is possible that the clarity of some results may be affected by problems associated with the administration of the DNA vaccine. Indeed, much varied survival times may reflect different levels of immune response between different members of a given group.

Table 1

LEEP DNA immunisation and GBS challenge Experiment

Statistical analysis of survival times

	Mean Survival Times (hours)		
	UnVacc	3-60(ID-65)	3-5(ID-66)
1	27.583	54.416	42.916
2	27.583	31.000	42.916
3	24.583	43.000	32.874
4	22.250	34.916	42.916
5	35.916	38.958	27.333
6	22.250	34.916	30.916
Mean	**27.583**	**40.458**	**37.791**
sd	**5.1691**	**8.9959**	**7.2860**
p value		**0.0098**	**0.0215**

p value refers to statistical significance when compared to unvaccinated controls.

Comment

ID-65 (3-60)

Mice immunised with the '3-60 (ID-65)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

ID-66 (3-5)

Mice immunised with the '3-5 (ID-66)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

Example 3

Expression and Screening Group B Streptococcal LEEP derived Proteins in Protein vaccination experiments.

Expression of proteins

Prioritised genes ie, those selected on the basis of predicted expression features as deduced from sequence characteristics (as described in Figure 1), were cloned and expressed as recombinant proteins using the pET system (Novagen, Inc., Madison, WI) utilising *Escherichia coli* as a host. Target genes were cloned into the pET28b(+) plasmid expression vector. The pET28b(+) vector is designed for high level expression and purification of target proteins. This vector carries a T7 promoter for transcription of a target gene, followed by an N-terminal His•Tag®/thrombin/T7•Tag® configuration, a multi-cloning site containing unique restriction enzyme sites for cloning purposes, and an optional C-terminal His•Tag sequence. The vector also carries a kanamycin resistance gene for selection purposes and for maintaining target gene expression (pET System Manual, 8th edition, Novagen).

Preparation of protein vaccines

Oligonucleotide primers were designed for each individual target gene derived using the LEEP system unless stated otherwise. Each gene was examined thoroughly. Where possible primers were designed so that they would target that part of the gene predicted to encode only the mature portion of the protein (APPENDIX II). It is hoped that expressing those corresponding to the predicted mature protein only, might facilitate its correct folding when finally expressed *in vitro*. Oligonucleotide primers were designed so that sequences, encoding the putative N-terminal signal peptide of the target protein, would not be included in the final amplification product to be cloned pET28b(+). The signal peptide directs the polypeptide precursor to the cell membrane via the protein export pathway where it is normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein). Hence the signal peptide would not be expected to form any part of the mature target protein, whether it be displayed on the bacterium's surface or secreted. For this purpose, classical signal peptides and their cleavage sites were predicted using the DNA Strider™ Program (CEA, France) and the SignalP V1.1 program, which predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms (Nielsen *et al., Protein Engineering* **10**: 1-6 (1997)). Where a N-terminal leader peptide sequence was not obvious, primers were designed to include the whole of the gene sequence for cloning and expression.

All oligonucleotide primers were designed to incorporate appropriate restriction enzyme sites to facilitate cloning into the pcDNA3.1 MCS region (APPENDIX II). Forward primers included an *Nco* I (5'-ccatgg-3') or *Nhe* I (5'-gctagc-3') restriction enzyme site and an 'ATG' start codon in-frame with the target gene open reading frame (orf). All reverse primers included a *Not* I restriction enzyme site 5' -gcggccgc-3' and were designed so that the target gene could be expressed in frame with the C-terminal His•Tag (i.e. the stop codon of the target gene was not

included). Using the *Nco* I and *Not* I, allowed the removal of the N-terminal His•Tag®, thrombin and T7•Tag® DNA sequences. At the same time target genes were cloned immediately downstream of a highly efficient ribosome binding site (from the phage T7 major capsid protein), to facilitate high level expression/translation of the target gene by T7 RNA polymerase, and subsequent purification by means of the C-terminal His•Tag. All target gene-specific forward and reverse primers were designed with compatible melting temperatures to facilitate their amplification.

All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template using Vent DNA polymerase (NEB) using conditions recommended by the manufacturer. A typical amplification reaction involved an initial denaturation step at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). This was followed by a final extension period at 72°C for 10 minutes. All PCR amplified products were extracted once with phenol:chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). Purified target gene DNA amplicons were then digested *Nco* I (or *Nhe* I) and *Not* I restriction enzymes, and cloned into *Nco* I and *Not* I digested pET28b(+) plasmid vector using *E. coli* DH5α or *E. coli* BL21 (DE3) as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping.

Determination of target protein expression and solubility

Glycerol stocks of *E. coli* BL21 DE3 pET28b(+) strains expressing recombinant proteins were used to inoculate 10 ml Luria broth containing Kanamycin (30 μg/ml) which were grown overnight at 37°C with vigorous shaking (300 rpm).

A 20-40 ml Luria broth containing Kanamycin (30 μg/ml) was inoculated with 1:100 dilution of the overnight culture from step 1 and grown at 37°C with vigorous shaking (300 rpm). When the culture reached an OD_{600} of between 0.6 and 1.0, IPTG was added to a final concentration of 1mM. Typically cultures were induced for 3 hours. Cells were then harvested by centrifugation at 7000 g for 10 min. The cell pellet was then resuspended in 1/10 volume of lysis buffer (50mM NaH_2PO_4, pH.8.0; 300mM NaCl;10mM imidazole; 10% glycerol). Lysozyme was then added to a final concentration of 1mg/ml, and the suspension was incubated on ice for 30 min. The suspension was then sonicated on ice (six 10-sec bursts at 200-300 W with a 10-sec cooling period. The lysate was then centrifuged at 10,000g for 20 min. The supernatant (containing soluble protein) was transferred to a sterile 2 ml eppendorf. The pellet was resuspended in 2 ml of solubilisation buffer (8 M Urea; 50mM NaH_2PO_4, pH.8.0; 300mM NaCl; 10% glycerol). This suspension contained the insoluble protein fraction. Aliquots from both the soluble and insoluble fractions were transferred to new eppendorfs. The protein samples were denatured by adding an equal volume of 2x SDS-PAGE buffer and heating at 95°C for 5 min. Denatured extract samples were then analysed by SDS-PAGE to determine target gene expression and solubility.

Large scale expression of recombinant target proteins

Glycerol stocks of *E. coli* BL21 DE3 pet28b(+) strains expressing recombinant proteins were used to inoculate 10 ml Luria broth containing Kanamycin (30 μg/ml) which were grown overnight at 37°C with vigorous shaking (300 rpm). 5 ml of an overnight culture of a recombinant strain was used to inoculate a 250 ml Luria broth containing kanamycin (30 μg/ml) which was grown at 37°C with vigorous shaking (300 rpm). When the culture reached an OD_{600} of between 0.6 and 1.0, IPTG was added to a final concentration of 1mM. Typically, cultures were induced for 3 hours. Cultures were then centrifuged to a pellet and stored frozen at –20°C.

Purification of target antigens.

Ni-NTA agarose (Qiagen LTD, West Sussex, UK; Cat. No. 30210) was used to purify the His-Tagged recombinant proteins. The 6xHis affinity tag which was expressed in frame with the target proteins in pET28b(+), facilitates binding to Ni-NTA. Ni-NTA offers high binding capacity (with minimal non-specific binding) and can bind 5-10 mg of 6xHis-tagged protein per ml of resin. The 6xHis-tag is poorly immunogenic, and at pH 8.0, the tag is small, uncharged and therefore does not generally interfere with the structure and function of the protein (The QIA*expressionist*, Qiagen Handbook, March 1999).

NOTE: All the proteins (LEEP-derived, unless stated otherwise) described here were purified under denaturing conditions except ID-65. ID-65 was prepared and purified under native conditions.

Purification under native conditions

The frozen pellet was allowed to thaw on ice for 15 minutes and then resuspended in 10 ml of lysis buffer (50mM NaH_2PO_4, pH.8.0; 300mM NaCl;10mM imidazole;

10% glycerol). Lysozyme was then added to a final concentration of 1mg/ml, and the suspension was incubated on ice for 30 min. The suspension was then sonicated on ice (six 10-sec bursts at 200-300 W with a 10-sec cooling period0. Dnase I (5 μg/ml) was then added to the lysate, which was then incubated on ice for 10-15 min. The lysate was then centrifuged at 10,000 rpm for 20 min at 4°C to pellet cell debris. The clear lysate supernatant was then loaded into a polypropylene column (Qiagen; Cat. No. 34964), bottom cap attached. 1.5 ml of 50% Ni-NTA was then added, the column sealed and the suspension was allowed to mix gently using a rotating wheel for 1-2 hours at 4°C. The column containing the lysate/Ni-NTA mix was then placed upright using a retort stand, and the Ni-NTA was allowed to settle. The bottom cap was removed and the lysate was allowed to flow through. The column was then washed with three to six 4 ml volumes of wash buffer (50mM NaH_2PO_4, pH.8.0; 300mM NaCl;20mM imidazole; 10% glycerol). The protein was then eluted in 0.5 ml aliquots of elution buffer (50mM NaH_2PO_4, pH.8.0; 300mM NaCl;500mM imidazole; 10% glycerol). Eluate fractions were then analysed by SDS-PAGE and those containing the protein were pooled and dialysed against a PBS (pH 7.0)-glycerol (10%) solution.

Purification and refolding under denaturing conditions

The frozen pellet was allowed to thaw on ice for 15 minutes and then resuspended in 10 ml of buffer containing 8 M Urea, 300 mM NaCl, 10% glycerol, 0.1 M NaH_2PO_4, pH.8.0, and 10 mM imidazole. The cells were then lysed by gentle vortexing for 1 hour at room temperature. The lysate was then centrifuged at 10,000g for 20 minutes to pellet cellular debris. The clear lysate supernatant was then loaded into a polypropylene column (Qiagen; Cat. No. 34964), bottom cap attached. 1.5 ml of 50% Ni-NTA slurry was then added, the column sealed and the suspension was allowed to mix gently using a rotating wheel for 1-2 hours at room

temperature. The column containing the lysate/Ni-NTA mix was then placed upright using a retort stand, and the Ni-NTA was allowed to settle. The bottom cap was removed and the lysate was allowed to flow through. The column was then washed with 4-8 ml of buffer containing 8 M Urea, 300 mM NaCl, 10% glycerol, 0.1 M NaH_2PO_4, pH 8.0, and 10 mM imidazole. The resin was then washed with a gradient of 6 to 0 M in a buffer containing 0.1 M NaH_2PO_4, pH.8.0, 300 mM NaCl and 10% glycerol to facilitate the slow removal of urea and gradual refolding of target protein. The resin was then washed with a buffer containing 0.1 M NaH_2PO_4, pH 7.0, 500 mM NaCl and 10% glycerol. The recombinant protein was then eluted in 0.5 ml aliquots with 500 mM Imidazole in 0.1 mM NaH_2PO_4, pH 7.0, 500 mM NaCl and 10% glycerol. The fractions were analysed on SDS-PAGE and those containing the protein were pooled and dialysed against a PBS (pH 7.0)–glycerol (10%) solution.

All purified proteins were analysed by SDS-PAGE, as shown in Figures 5, 6 and 7, prior to their use as antigens in immunisation and vaccination experiments.

Protein Vaccinations

Vaccines were composed of the target protein in phosphate buffered saline/10% glycerol and mixed with aluminium hydroxide (alum) (Imject®Alum, Pierce, Rockford, Ill.). Each dose (unless otherwise stated) of vaccine contained 25 μg of purified protein in 50 μl of PBS/10% glycerol, mixed with 50 μl of alum. Groups of 6-8 CBA/ca mice (Harlan, UK) were immunised subcutaneously with the vaccines and again 4 weeks later. A control group received 100 μl dose of PBS/10% glycerol with alum. All vaccinated groups consisted of 6 mice. Mice were challenged at 7 weeks (unless otherwise stated). Mice were injected intraperitoneally (i.p.) with between 2.5-5 X 10^6 bacteria diluted in 0.5 ml Todd-Hewitt broth. Deaths were recorded daily for 7 days. The challenged mice were observed daily for signs of illness. Typical symptoms in an appropriate order included piloerection, an

increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death.

Analysis of antibody responses

Mice (6 per group) were immunised with two doses of vaccine with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. Total Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the original purified protein as the coating antigen.

Standard ELISA protocol

Solutions

Carbonate/bicarbonate buffer, pH 9.8

0.80g Na_2CO_3

1.46g $NaHCO_3$

pH to 9.6 using HCl

Add distilled water (dH_2O) to a final volume of 500ml.

n-NITROPHENYL PHOSPHATE SUBSTRATE

Diethanolamine Buffer, pH 9.8

48.5 ml diethanolamine

pH to 9.8 using 1M HCl

Add dH_2O to a final volume of 500ml

NOTE: ELISAs were optimised for each protein submitted for immunisation.

PROTOCOL

1. ELISA plates (Greiner labortechnik 96 well plates: Cat. No. 655061) with an appropriate concentration of recombinant protein diluted in carbonate/bicarbonate buffer (50 μl/well). Cover plates with plastic or foil and leave overnight at 4°C.
2. Quickly wash plates twice in a tub/container containing PBS/0.05%Tween-20 and then pat dry.
3. Block plates with 3% BSA in PBS/Tween (100μl /well) for 1 hour at room temperature.
4. Wash the plates 3 times PBS/Tween as before and pat dry as before.
5. Apply (primary antibody) protein-specific antiserum (50μl/well) diluted from 1/50 in a doubling dilution series in PBS/Tween and incubate at room temperature for 90 minutes.
6. Wash plates as before (3 times quickly), followed up by 2 X 3 minute soaks (in PBS/Tween)
7. Apply diluted secondary antibody alkaline phosphatase conjugate. For anti-mouse Total IgG alkaline phospatase conjugate (Goat Anti-Mouse IgG-AP, Southern Biotechnology Associates,Birmingham, AL. Cat. No. 1030-04) dilute 1/3000 in PBS/Tween and apply 50 μl per well and incubate at room temperature for 90 minutes.
8. Wash plates as in step 6.

9. Apply substrate. Dissolve one 5mg tablet of nitrophenyl phosphate (Sigma:kept in freezer) in 5ml of diethanolamine buffer. Apply 100 μl per well. Cover with foil (a light-sensitive reaction) and leave at room temperature for 30 minutes. Read Optical densities (OD) at a wavelength of 405nm.
10. Plot curves of OD Vs dilution (log scale). Calculate end-point titres as the dilution giving the same OD as the mean of the OD obtained from the wells containing the 1/50 dilution of pre-immune serum.

ELISA Plate format

2°	1/50	1/100	1/200	1/400	1/800	1/1600	1/3200	1/6400	1/12800	1/25600	1/51200
1°	Duplicate										
Pre											
Pre											
Pre											

Table Summary

Pre Replicate wells of pooled pre-inoculation serum (50μl per well) diluted to 1/50 are included on every plate in order for end point titres to be calculated.

2° Is a blank control well to which no secondary antibody conjugate is applied. PBS/Tween by itself is applied instead

1° Is a blank control well to which no primary antibody is applied. PBS/Tween by itself is applied instead

Duplicate Each serum is analysed in duplicate

The dilution series used is indicated (see first row of table). Beginning with a 1/50 dilution, sera are diluted two-fold in PBS/Tween in doubling dilution series as indicated.

Protein Immunisation data

ID-65 and ID-83

The ID-65 and ID-83 vaccines were composed of the target proteins in phosphate buffered saline/10% glycerol mixed with aluminium hydroxide (alum)

(Imject®Alum, Pierce, Rockford, Ill.). Each dose of vaccine contained 20 μg of purified protein in 100 μl of PBS/10% glycerol, mixed with 50 μl of alum. A group of 6-8 week old CBA/ca mice (Harlan, UK) were immunised subcutaneously with the ID-65 and ID-83 vaccine and again 4 weeks later. A control group received a 150 μl dose of PBS/10% glycerol (2:1) with alum. All groups consisted of 6 mice. Mice were tail bled at 5 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-65 and ID-83 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the purified protein as the coating antigen. ELISA was also performed using sera obtained at 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.

NOTE: ELISA plates were coated with the ID-65 or ID-83 proteins at a concentration of 1 μg/ml.

Protein Vaccination -ELISA results for ID-65 and ID-83

Mice (6 per group) were immunised with two doses of the ID-65 and ID-83 vaccines with a four week interval. Mice were tail bled at 5 weeks post primary vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-65 and ID-83 proteins as the coating antigen. Subsequent to optimisation, ELISA plates were coated at a concentration 1ug/ml for both the purified ID-65 and ID-93 proteins. Total IgG titres were measured against pre-immune serum (1/50 dilution). The results are shown in Table 2 and graphically in Figure 8.

Table 2

Serum (Group)	ID-65+Alum (n=6)	PBS+Alum (n=6)	ID-83+Alum (n=6)	PBS+Alum (n=6)
Coating antigen	ID-65		ID-83	
Bleed	5 weeks	5 weeks	5 weeks	5 weeks
Total IgG Titres (mouse 1-6)	7535763	965	82081	61
	1557649	90	50027	50
	3319737	108	154670	80
	1832259	176	57901	96
	8794360	371	66497	125
	1445728	0	49928	0
Average	4080916	285	76851	69
Standard Deviation	3258818	355	39985	43

Protein Immunisation and Challenge data (ID-93)

ID-93

The ID-93 vaccine was composed of the target ID-93 protein in phosphate buffered saline/10% glycerol mixed with aluminium hydroxide (alum) (Imject®Alum, Pierce, Rockford, Ill.). Each dose of vaccine contained 25 μg of purified protein in 100 μl of PBS/10% glycerol, mixed with 100 μl of alum. A group of 6-8 week old CBA/ca mice (Harlan, UK) were immunised subcutaneously with the ID-93 vaccine and again 4 weeks later. A control group received PBS/10% glycerol with alum. Both groups consisted of 6 mice. Mice were challenged at 7 weeks (unless otherwise stated). Mice were injected intraperitoneally (i.p.) with 5 X 10^6 bacteria diluted in

0.5 ml Todd-Hewitt broth. The challenged mice were observed daily for signs of illness. Deaths were recorded daily for 7 days. Survival data are shown in Table 3 and graphically in Figure 9.

Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-93 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the pure ID-93 protein as the coating antigen. ELISA was also performed using sera obtained at 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.

Note: ELISA plates were coated with the ID-93 protein at a concentration of 1 μg/ml.

Table 3

ID-93 protein immunisation and GBS challenge experiment

Statistical analysis of Survival Times

Group	PBS+Alum	ID-93+Alum
Survival Times (hours)	22.37	29.37
	22.37	35.12
	15.37	32.62
	28.03	32.62
	29.53	37.12
	26.53	27.87
Mean	24.03	32.45
sd	5.16	3.45
p value		0.01

p value refers to statistical significance when compared to unvaccinated controls.

Comment

ID-93 (RS-70)

Mice immunised with the ID-93-Alum vaccine exhibited significantly longer survival times when compared with the PBS-Alum control group.

(Statistical Significance was determined by the Mann-Whitney U test using a 95% confidence level ($p > 0.05$).

Protein Vaccination -ELISA results for ID-93

Mice (6 per group) were immunised with two doses of the ID-93 vaccine with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-93 protein as the coating antigen. Subsequent to optimisation, ELISA plates were coated with the purified ID-93 protein at a concentration of 1 μg/ml. Total IgG titres were measured against pre-immune serum (1/50 dilution). The results are shown in Table 4 and graphically in Figure 10.

Table 4

Serum Group	ID-93+Alum(n=6)		PBS/10%glycerol (n=6) (control)	
Coating antigen	ID-93	ID-93	ID-93	ID-93
Bleed	3 weeks	6 weeks	3 weeks	6 weeks
Total IgG Titres (mouse 1-6)	87196	3000000	39	100
	99544	8000000	31	16
	19620	2000000	31	79
	34724	10000000	59	48
	59990	10000000	24	328
	30041	4000000	13	40
Average	55186	6166667	33	102
Standard error	32654	3600926	15	115

Protein Immunisation data
ID-89 and ID-96

The ID-89 and ID-96 vaccines were composed of the target proteins in phosphate buffered saline/10% glycerol mixed with TitreMax Gold adjuvant (Sigma, Missouri, USA) according to the manufacturers instructions. The ID-89 vaccine contained 25 μg of purified protein 50 μl of PBS/10% glycerol, mixed with 50 μl of TitreMax Gold. The ID-96 vaccine contained 12.5 μg of purified protein 50 μl of PBS/10% glycerol, mixed with 50 μl of TitreMax Gold. Groups of 6-8 week old CBA/ca mice (Harlan, UK) were immunised subcutaneously with the ID-89 and ID-96 vaccines and again 4 weeks later. A control group received a 100 μl dose PBS/10% glycerol with TitreMax Gold (1:1). Both groups consisted of 6 mice. Mice were tail bled at 3

weeks and 6 weeks post primary vaccination to obtain sera. The presence of total Immunoglobulin G (IgG) antibodies to the ID-65 and ID-83 protein in sera was determined by enzyme-linked immunosorbent assay (ELISA), using the purified protein as the coating antigen. ELISA was also performed using sera obtained at 3 weeks and 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group.

Note: ELISA plates were coated with the ID-89 or ID-96 proteins at a concentration of 1 μg/ml and 3 μg/ml respectively.

Protein Vaccination -ELISA results for ID-89 and ID-96

Mice (6 per group) were immunised with two doses of the ID-89 and ID-96 vaccines with a four week interval. Mice were tail bled at 3 weeks and 6 weeks post primary vaccination to obtain sera. The Immunoglobulin G (IgG) titres to the vaccine protein component in sera were determined by enzyme-linked immunosorbent assay (ELISA), using the purified ID-65 and ID-83 proteins as the coating antigen. Subsequent to optimisation, ELISA plates were coated with purified ID-89 and ID-96 protein at a concentration 1ug/ml and 3 μg/ml respectively. Total IgG titres were measured against pre-immune serum (1/50 dilution). ELISA was also performed on both proteins using sera obtained at 3 weeks and 6 weeks post-primary vaccination from the PBS/10% glycerol immunised control group. Results are shown in tables 5a and 5b and graphically in Figure 11.

Table 5a

Serum	ID-89+TitreMax Gold (n=6)		ID-96+TitreMax Gold(n=6)	
Coating antigen	ID-89		ID-96	
Bleed	3 weeks	6 weeks	3 weeks	6 weeks
Total IgG Titres (mouse 1-6)	146940	1000000	190371	10000000
	89672	1000000	212505	10000000
	173532	2000000	167613	5000000
	85161	751210	110378	5000000
	88956	551281	142614	1000000
	27880	2000000	191085	1000000
Average	102024	1217082	169094	5333333
Standard Deviation	51451	629364	37341	4033196

Table 5b

Serum	PBS/10%glycerol (n=6)		PBS/10%glycerol (n=6)	
Coating protein	ID-89		ID-96	
Bleed	3 weeks	6 weeks	3 weeks	6 weeks
Total IgG Titres (mouse 1-6)	3	7	33	31
	0	1	77	62
	29	31	77	1
	34	4	52	29
	0	2	125	31
	5	4	113	0
Average	13	11	80	26
Standard deviation	15	12	35	23

Example 4

Conservation and variability of candidate vaccine antigen genes among different isolates of Group B Streptococci

An initial Southern blot analysis was carried out to determine cross-serotype conservation of novel Group B Streptococcal genes isolated using the LEEP system unless stated otherwise. Analysing the serotype distribution of a target gene will also determine their potential use as antigen components in a GBS vaccine. The Group B Streptococcal strains whose DNA was analysed as part of this study are listed in APPENDIX III

Amplification and labelling of specific target genes as DNA probes for Southern blot analysis.

Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system unless stated otherwise. The same primers already described in APPENDIX II were used to amplify corresponding gene-specific DNA probes. Specific gene targets were amplified by PCR using Vent DNA polymerase (NEB) according to the manufacturers instructions. Typical reactions were carried out in a 100 μl volume containing 50 ng of GBS template DNA, a one tenth volume of enzyme reaction buffer, 1 μM of each primer, 250 μM of each dNTP and 2 units of Vent DNA polymerase. A typical reaction contained an initial 2 minute denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). The annealing temperature was determined by the lower melting temperature of the two oligonucleotide primers. The reaction was concluded with a final extension period of 10 minutes at 72°C.

All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). For use as DNA probes, purified amplified gene DNA fragments were labelled with digoxygenin using the DIG Nucleic Acid Labelling Kit (Boehringer Mannheim) according to the manufacturer's instructions.

Southern blot hybridisation analysis of Group B Streptococcal genomic DNA

Genomic DNA had previously been isolated from all strains of Group B Streptococci which were investigated for conservation of LEEP-derived (unless stated otherwise) gene targets. Appropriate DNA concentrations were digested using either *Hin* DIII or *Eco* RI restriction enzymes (NEB) according to manufacturer instructions and analysed by agarose gel electrophoresis. Following agarose gel electrophoresis of DNA samples, the gel was denatured in 0.25M HCl for 20 minutes and DNA was transferred onto Hybond^{TM} N^+ membrane (Amersham) by overnight capillary blotting. The method is essentially as described in Sambrook *et al.* (1989) using Whatman 3MM wicks on a platform over a reservoir of 0.4M NaOH. After transfer, the filter was washed briefly in 2x SSC and stored at 4°C in Saran wrap (Dow chemical company).

Filters were prehybridised, hybridised with the digoxygenin labelled DNA probes and washed using conditions recommended by Boehringer Mannheim when using their DIG Nucleic Acid Detection Kit. Filters were prehybridised at 68°C for one hour in hybridisation buffer (1% w/v supplied blocking reagent, 5x SSC, 0.1% v/v N-lauryl sarcosine, 0.02% v/v sodium dodecyl sulphate[SDS]). The digoxygenin labelled DNA probe was denatured at 99.9°C for 10 minutes before being added to the hybridisation buffer. Hybridisation was allowed to proceed overnight in a rotating Hybaid tube in a Hybaid Mini-hybridisation oven. Unbound probe was removed by washing the filter twice with 2x SSC- 0.1% SDS for 5 minutes at room

temperature. For increased stringency filters were then washed twice with 0.1x SSC-0.1% SDS for 15 minutes at 68°C. The DIG Nucleic Acid Detection Kit (Boehringer Mannheim) was used to immunologically detect specifically bound digoxygenin labelled DNA probes.

Results of Southern blot analysis

Unless otherwise stated, all genomic digests and their corresponding Southern blots followed an identical lane order as described in Table 6 below.

Table 6

Lane	1	2	3	4	5	6	7
Strain	1 kb molecular Weight Marker	515	A909	SB35	H36B	18RS21	1954/92
Serotype		Ia	Ia	Ib	Ib	II	II

Lane	8	9	10	11	12	13	14
Strain	118/158	97/0057	BS30	M781	97/0099	3139	1169-NT
Serotype	II	II	III	III	III	IV	V

Lane	15	16	17	18	19	20
Strain	GBS 6	7271	JM9	Group A Strepococcus	*Streptococcus pneumoniae*	1 kb molecular
Serotype	VI	VII	VIII	_	14	Weight Marker

For comparative purposes, it was decided to analyse the serotype distribution of the GBS *rib* gene, which encodes the known protective immunogen Rib. Rib has previously been shown to be present in serotype III and some strains of serotype II but not in serotypes Ia or Ib (Stalhammar-Carlemalm *et al.*, *J. Exp. Med.* **177**: 1593-1603 (1993)).

Confirmation of this pattern would not only give increased confidence in interpreting subsequent results, it would also determine if a *rib* gene homologue was present in the remaining GBS serotypes being investigated here. Primers designed for the amplification of *rib* for use as a gene probe in Southern blot analysis are described in APPENDIX II.

Table 7 – Lane order for Figure 12 (*rib* gene Southern blot analysis)

Lane	1	2	3	4	5	6	7
strain	1 kb molecular	515	A909	SB35	H36B	18RS21	1954/92
serotype	Weight Marker	Ia	Ia	Ib	Ib	II	II

Lane	8	9	10	11	12	13	14
strain	118/158	97/0057	BM110	BS30	M781	97/0099	3139
serotype	II	II	III	III	III	III	IV

Lane	15	16	17	18	19	20
strain	1169-NT	GBS 6	7271	JM9	Group A Strepococcus	*Streptococcus pneumoniae*
serotype	V	VI	VII	VIII	_	14

Rib (Figure 12) Comment

The Southern blot analysis shown in Figure 12 indicates that the *rib* gene is not conserved across all GBS serotypes. *rib* appears to be absent from all serotype Ia and Ib strains (lanes 2 to 5) and from strains 118/158 and 97/0057 of serotype II (lanes 8 and 9). However, *rib* would appear to present in strains 18RS21 and 1954/92 of serotype II (lanes 6 and 7) and in all strains of serotype III (lanes 10 to 13). This is in agreement with previously published data (Stalhammar-Carlemalm *et al.*, 1993 [*supra*]). *rib* would also appear to be present in strains representing serotypes VII and VII (lanes 17 and 18) but was absent from strains representing serotypes IV, V and V (lanes 14 to 16) as well as the control strains (lanes 19 and 20). The *rib* gene probe did hybridise with lower intensity to genomic DNA fragments from strains representing serotypes Ia, Ib, IV, VI, VII and serotype II strains 118/158 and 97/0057. This may indicate the presence of a gene in these strains with a lower level of homology to *rib*. These hybridising DNA fragments may contain a homologue of the GBS *bca* gene encoding the Ca protein antigen which has been shown to be closely homologous to the Rib protein (Wastfelt *et al.*, *J. Biol. Chem.* **271**:18892-18897 (1996)). If this is the case, it would be in agreement with previous work which showed all strains of serotypes Ia, Ib, II and III to be positive for one the two proteins (Stalhammar-Carlemalm *et al.*, 1993 [*supra*]). However, the apparent variable distribution of the *rib* gene amongst different GBS

serotypes, makes it a less than ideal candidate for use in a GBS vaccine that is cross-protective against all serotypes.

ID-65 (Figure 13) Comment

The Southern blot analysis described in Figure 13 indicates that gene ID-65 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 3.0 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-65 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level. The ID-65 DNA probe also hybridised weakly to the 1.636 bp molecular weight marker (the 1 kb DNA ladder from NEB was used to estimate DNA fragment sizes in all Southern blot analyses).

ID-89 (Figure 14) Comment

The Southern blot analysis described in Figure 14 indicates that gene ID-89 may not be conserved across all GBS serotypes. A 4.0 kb *Hin*DIII-digested genomic DNA fragment from 12 out of 16 GBS strains hybridised specifically to the ID-89 gene probe. In addition, a 3.25 kb *Hin*DIII-digested genomic DNA fragment from the GBS strain Ib (SB35) [lane 4] also hybridised specifically with the ID-89 gene probe. However, the ID-89 gene probe did not hybridise to digested genomic DNA fragments from strains Ia (515) [lane 2], IV (3139) [lane 13] and V (1169-NT) [lane 14], suggesting that these strains do not possess a ID-89 gene homologue.

ID-93 (Figure 15) Comment

The Southern blot analysis described in Figure 15 indicates that gene ID-93 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin*

DIII-digested genomic DNA fragment of approximately 3.25 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-93 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level.

ID-96 (Figure 16) Comment

The Southern blot analysis described in Figure 16 indicates that gene ID-96 is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 12.0 kb in DNA digests from all GBS representatives, and was absent from both the control strains (lanes 18 and 19). This would suggest that the ID-96 gene is conserved across all GBS serotypes (and strains) at both the gene and locus level.

APPENDIX I

ID-65

Forward Primer

5' – cggatccgccaccatgGCGGATCAAACTACATCGGTTC - 3'

Reverse Primer

5' - ttgcggccgcGTTGGGATAACTAGTCGGTTTAGTCG

Length (including restriction sites) = 1541bp

Incorporating 1515bp of gene-specific sequence encoding 505 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

Sequence predicted to encode a signal peptide was omitted from amplified product

ID-66

Forward Primer

5' – cggatccgccaccatgAATCTTTATTTCCATAGTACTCCCTTGC - 3'

Reverse Primer

5' – ttgcggccgcAAAATGATCAGTTTGAGGGTAAAAGAG - 3'

Length (including restriction sites) = 767bp

Incorporating 747bp of gene-specific sequence encoding 247 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

Sequence predicted to encode a signal peptide was omitted from amplified product

APPENDIX II

ID-65

Forward Primer

5' – catgccatgGCGGATCAAACTACATCGGTTC - 3'

Reverse Primer

5' - ttgcggccgcGTTGGGATAACTAGTCGGTTTAGTCG

Length (including restriction sites) = 1534bp

Incorporating 1515bp of gene-specific sequence encoding 505 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 60°C

ID-83

Forward Primer

5' – catgccatggcaAAAATAGTAGTACCAGTAATGCCTC – 3'

ReversePrimer

5' – ttgcggccgcCTCTGAAATAGTAATTTGTCCG – 3'

Length (including restriction sites) = 626bp

Incorporating 624bp of gene-specific sequence encoding 208 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 52°C

ID-89

Forward Primer

5' – catgccatgggaAAGAAAGCAAATAATGTCAGTCC – 3'

Reverse Primer

5' – ttgcggccgcATTGGGTGTAAGCATTTTTTC –3'

Length (including restriction sites) = 990bp

Incorporating 969bp of gene-specific sequence encoding 323 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 54°C

ID-93

Forward Primer

5' – catgccatgggaACTGAGAACTGGTTACATACTAAAG – 3'

ReversePrimer

5' – ttgcggccgcATTAGCTTTTTCAACAATTTCTC – 3'

Length (including restriction sites) = 759bp

Incorporating 744bp of gene-specific sequence encoding 248 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 51°C

ID-96

Forward Primer

5' – ctagctagccgATGTTTGCGTGGGAAAG – 3'

ReversePrimer

5' – ttgcggccgcATAAGATTTAACAATACCAAGTAATATAGC – 3'

Length (including restriction sites) = 944bp

Incorporating 921bp of gene-specific sequence encoding 307 amino acids of the putative mature protein.

Annealing temperature for PCR amplification = 53°C

rib (control)
Forward primer

5' - ggggtaccggccaccATGGCTGAAGTAATTTCAGGAAGT -3'

Reverse primer

5' - cggaattccgTTAATCCTCTTTTTTTCTTAGAAACAGAT

Length (including restriction sites) = 3559bp

Incorporating 3531bp of gene-specific sequence encoding 1177 amino acids of the mature protein.

Annealing temperature for PCR amplification = 55°C

APPENDIX III

Listed below are the details (serotype and strain designation) of Group B Streptococcus strains whose DNA was analysed for gene conservation

	SEROTYPE	**STRAIN**
	Ia	515
	Ia	A909
	Ib	SB35
	Ib	H36B
	II	18RS21
	II	1954/92
	II	118/158
	II	97/0057
	III	BM110
	III	BS30
	III	M781
	III	97/0099
	IV	3139

V	1169/NT
VI	GBS VI
VII	7271
VIII	JM9

A group A Streptococcal strain (serotype M1, strain NCTC8198) and *Streptococcus pneumoniae* (serotype 14) were also included in the analysis for control purposes.

CLAIMS

1. A Group B Streptococcus polypeptide or protein having a sequence selected from those described in fig 1, or fragments or derivatives thereof.

2. Derivatives or variants of the proteins, polypeptides, and peptides as claimed in claim 1 which show at least 50% identity to those proteins, polypeptides and peptides claimed in claim 1.

3. A Group B Streptococcus polypeptide or protein, or derivative or variant thereof, as claimed in claim 1 or claim 2 , which is isolated or recombinant.

4. A nucleic molecule comprising or consisting of a sequence which is:

(i) any of the DNA sequences set out in figure 1 herein or their RNA equivalents;

(ii) a sequence which is complementary to any of the sequences of (i);

(iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);

(iv) a sequence which shows substantial identity with any of those of (i), (ii) and (iii); or

(v) a sequence which codes for a derivative, or fragment of a nucleic acid molecule shown in figure 1.

5. A vector comprising one or nucleic acid molecules as defined in claim 4.

6. A vector as claimed in claim 4 further comprising nucleic acid encoding any one or more of the following: promoters, enhancers, signal sequences, leader sequences, translation start and stop signals, DNA stability controlling regions, or a fusion partner.

7. The use of a vector as claimed in claim 5 or claim 6 in the transformation or transfection of a prokaryotic or eukaryotic host.

8. A host cell transformed with a vector as defined in claim 5 or claim 6..

9. A process for producing a Group B Streptococcus polypeptide or protein, or derivative or variant thereof, as claimed in claim 1 or claim 2, the process comprising expressing the polypeptide or protein in a host cell as claimed in claim 8.

10. An antibody, an affibody, or a derivative thereof which binds to one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as defined in any one of claims 1 to 3.

11. An immunogenic composition comprising one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof as defined in any one of claims 1 to 3.

12. An immunogenic composition as claimed in claim 11 wherein the proteins, polypeptides, peptides, or fragments or derivatives thereof include ID-65 or ID-83, ID-89, ID-93 or ID-96.

13. An immunogenic composition as claimed in claim 11 or claim 12 which is a vaccine.

14. An immunogenic composition comprising one or more of the nucleic acid sequences as defined in claim 4.

15. An immunogenic composition as claimed in claim 14 wherein the nucleic acid sequences include ID-65 or ID-66.

16. An immunogenic composition as claimed in claim 14 or claim 15 which is a vaccine.

17. Use of an immunogenic composition as defined in any one of claims 11 to 16 in the preparation of a medicament for the treatment or prophylaxis of Group B Streptococcus infection.

18. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as defined in claim 10.

19. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as defined in any one of claims 1 to 3.

20. A method of detection of Group B Streptococcus which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as defined in claim 4.

21. A kit for the detection of Group B Streptococcus comprising at least one antibody, affibody, or derivatives thereof as defined in claim 10.

22. A kit for the detection of Group B Streptococcus comprising at least one Group B Streptococcus protein, polypeptide, peptide, fragment or derivative thereof as defined in any one of claims 1 to 3.

23. A kit for the detection of Group B Streptococcus comprising at least one nucleic acid molecule as defined in claim 4.

24. A method of determining whether a protein, polypeptide, peptide, fragment or derivative thereof as defined in any one of claims 1 to 3 represents a potential anti-microbial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

FIG. 1

ID-65

Clone 3-60

GTGTTTATGATGAAAAAAGGACAAGTAAATGATACTAAGCAA
TCTTACTCTCTACGTAAATATAAATTTGGTTTAGCATCAGTAA
TTTTAGGGTCATTCATAATGGTCACAAGTCCTGTTTTTGCGGA
TCAAACTACATCGGTTCAAGTTAATAATCAGACAGGCACTAG
TGTGGATGCTAATAATTCTTCCAATGAGACAAGTGCGTCAAGT
GTGATTACTTCCAATAATGATAGTGTTCAAGCGTCTGATAAAG
TTGTAAATAGTCAAAATACGGCAACAAAGGACATTACTACTC
CTTTAGTAGAGACAAAGCCAATGGTGGAAAAAACATTACCTG
AACAAGGGAATTATGTTTATAGCAAAGAAACCGAGGTGAAAA
ATACACCTTCAAAATCAGCCCCAGTAGCTTTCTATGCAAAGAA
AGGTGATAAAGTTTTCTATGACCAAGTATTTAATAAAGATAAT
GTGAAATGGATTTCATATAAGTCTTTTGGTGGCGTACGTCGAT
ACGCAGCTATTGAGTCACTAGATCCATCAGGAGGTTCAGAGA
CTAAAGCACCTACTCCTGTAACAAATTCAGGAAGCAATAATC
AAGAGAAAATAGCAACGCAAGGAAATTATACATTTTCACATA
AAGTAGAAGTAAAAAATGAAGCTAAGGTAGCGAGTCCAACTC
AATTTACATTGGACAAAGGAGACAGAATTTTTTACGACCAAA
TACTAACTATTGAAGGAAATCAGTGGTTATCTTATAAATCATT
CAATGGTGTTCGTCGTTTTGTTTTGCTAGGTAAAGCATCTTCA
GTAGAAAAAACTGAAGATAAAGAAAAAGTGTCTCCTCAACCA
CAAGCCCGTATTACTAAAACTGGTAGACTGACTATTTCTAACG
AAACAACTACAGGTTTTGATATTTTAATTACGAATATTAAAGA
TGATAACGGTATCGCTGCTGTTAAGGTACCGGTTTGGACTGAA
CAAGGAGGGCAAGATGATATTAAATGGTATACAGCTGTAACT
ACTGGGGATGGCAACTACAAAGTAGCTGTATCATTTGCTGAC
CATAAGAATGAGAAGGGTCTTTATAATATTCATTTATACTACC
AAGAAGCTAGTGGGACACTTGTAGGTGTAACAGGAACTAAAG
TGACAGTAGCTGGAACTAATTCTTCTCAAGAACCTATTGAAAA
TGGTTTACCAAAGACTGGTGTTTATAATATTATCGGAAGTACT
GAAGTAAAAAATGAAGCTAAAATATCAAGTCAGACCCAATTT
ACTTTAGAAAAAGGTGACAAAATAAATTATGATCAAGTATTG
ACAGCAGATGGTTACCAGTGGATTTCTTACAAATCTTATAGTG
GTGTTCGTCGCTATATTCCTGTGAAAAAGCTAACTACAAGTAG
TGAAAAAGCGAAAGATGAGGCGACTAAACCGACTAGTTATCC
CAACTTACCTAAAACAGGTACCTATACATTTACTAAAACTGTA
GATGTGAAAAGTCAACCTAAAGTATCAAGTCCAGTGGAATTT
AATTTTCAAAAGGGTGAAAAAATACATTATGATCAAGTGTTA
GTAGTAGATGGTCATCAGTGGATTTCATACAAGAGTTATTCCG
GTATTCGTCGCTATATTGAAATTTAA

MFMMKKGQVNDTKQSYSLRKYKFGLASVILGSFIMVTSPVFADQTTSVQVNN
QTGTSVDANNSSNETSASSVITSNNDSVQASDKVVNSQNTATKDITTPLVETK
PMVEKTLPEQGNYVYSKETEVKNTPSKSAPVAFYAKKGDKVFYDQVFNKDN
VKWISYKSFGGVRRYAAIESLDPSGGSETKAPTPVTNSGSNNQEKIATQGNYT
FSHKVEVKNEAKVASPTQFTLDKGDRIFYDQILTIEGNQWLSYKSFNGVRRFV
LLGKASSVEKTEDKEKVSPQPQARITKTGRLTISNETTTGFDILITNIKDDNGIA
AVKVPVWTEQGGQDDIKWYTAVTTGDGNYKVAVSFADHKNEKGLYNIHLY
YQEASGTLVGVTGTKVTVAGTNSSQEPIENGLPKTGVYNIIGSTEVKNEAKISS
QTQFTLEKGDKINYDQVLTADGYQWISYKSYSGVRRYIPVKKLTTSSEKAKDE
ATKPTSYPNLPKTGTYTFTKTVDVKSQPKVSSPVEFNFQKGEKIHYDQVLVVD
GHQWISYKSYSGIRRYIEI*

Sequence description

A) Length: 1642 bp - 547 aa (full length gene)
B) Sequence Characteristics:
Potential leader peptide sequence
Orf is preceded by a potential Shine-
Dalgarno sequence.

ID-66

Clone 3-5

ATGATATTGAGACGTCGAACTATTGTTTTATGGCAACTGGGTATCGCCATT
TCTCTCATTCTTAGTATTCTAGCCTTAAATCTTTATTTCCATAGTACTCCCTT
GCAAACCAATGCAGCTTTACGGAACCTTGCTCCTTCATTAAACCATCTTTTT
GGGACAGATGGTTTAGGTAGGGATATGTTTGTCAGAACGATTAAAGGACT
TTATTTCTCTCTACAAGTCGGCTTATTAGGTGCCCTTATGGGGGTCATTCTG
GCGACAGTTTTTGGAGTGCTTGCAGGTTTAGGAAATAGCATTATTGATAAA
ATAATAGCATGGTTAGTTGATTTGTTTATTGGTATGCCTCATTTGATTTTTA
TGATTCTCATTTCTTTTGTTGTTGGGAAAGGTGCTCAAGGGGTCATCATTGC
AACGGCTGTTACACATTGGCCTTCTTTAGCAAGGCTTATCCGCAATGAAGT
CTATCATCTAAAGAATAAAGAATTTGTCCAACTTTCTAAAAGTATGGGAAA
AACGCCTTATTATATTGTGAGGCATCATATCCTGCCTTTGATTGCTTCTCAA
ATTTTCATTGGTTTTATCCTCTTATTTCCACATGTCATCCTACATGAAGCAT
CAATGACTTTCTTAGGATTTGGGCTCTCTGCCGAACAACCTTCGGTTGGTA
TCATTCTGTCAGAGGCAGCTAAGCATATCTCTCTTGGAAATTGGTGGTTGG
TTATCTTTCCAGGACTTTATCTTATTTTGGTTGTCAATGCATTTGATACTAT
CGGAGAATCTTTAAAGAAACTCTTTTACCCTCAAACTGATCATTTTTAG

FIG. 1CONT'D

MILRRRTIVLWQLGIAISLILSILALNLYFHSTPLQTNAALRNLAPSLNHLFGTD
GLGRDMFVRTIKGLYFSLQVGLLGALMGVILATVFGVLAGLGNSIIDKIIAWL
VDLFIGMPHLIFMILISFVVGKGAQGVIIATAVTHWPSLARLIRNEVYHLKNKE
FVQLSKSMGKTPYYIVRHHILPLIASQIFIGFILLFPHVILHEASMTFLGFGLSAE
QPSVGIILSEAAKHISLGNWWLVIFPGLYLILVVNAFDTIGESLKKLFYPQTDHF
*

Sequence description

A) Length: 822 bp - 274 aa (full length gene)
B) Sequence Characteristics:
Potential leader peptide sequence
Orf is preceded by a potential Shine-
Dalgarno sequence.

ID-78

Clone 3-5b

ATGACAGAAACATTATTAAGCATTAAAGACCTCTCCATCACCTTCACTCAA
TACGGAAGATTTTTAAAACCATTTCAATCAACACCGATACAAGCGCTGA
ATTTAGAAATTAAAAAAGGTGAGTTATTAGCTATTATAGGTGCTAGTGGTT
CGGGGAAGAGTTTATTAGCACATGCTATTATGGATATTCTTCCTAAAAATG
CATCTGTAACAGGAGATATGATTTATCGTGGTCAATCACTAAATTCTAAAC
GCATTAAACAGTTGCGAGGAAAAGATATTACGTTGATTCCACAATCAGTTA
ATTATTTAGATCCATCTATGAAAGTCAAACATCAGGTGCGCTTAGGTATCT
CAGAAAATTCAAAGGCTACTCAAGAAGGATTGTTTCAACAGTTTGGTTTAA
AAGAAAGTGATGGTGACTTGGATCCTTTCCAACTTTCTGGCGGAATGCTCC
GACGTGTTTTGTTTACAACGTGTATTAGTGATAAGGTTTCTTTGATTATTGC
GGATGAGCCCACCCCTGGATTACATCCAGATGCTCTGCAAATGGTTTTAGA
CCAACTACGCTCCTTTGCAGATAAAGGAATAAGCGTTATATTTATCACTCA
TGATATTGTAGCAGCTAGTCAAATTGCTGATCGTATTACTATTTTTAAAGA
GGGAAAAGCTATTGAAACAGCTCCAGCTAGTTTCTTTAGCGGAAATGGAG
AGCAGTTACAAACAGAATTTGCTAGAAGTTTATGGCGCTCTCTCCCACAGC
AAGAATTTTTGAAAGGAGTTACTCATGACCTTAGAGGCTAA

MTETLLSIKDLSITFTQYGRFLKPFQSTPIQALNLEIKKGELLAIIGASGSGKSLL
AHAIMDILPKNASVTGDMIYRGQSLNSKRIKQLRGKDITLIPQSVNYLDPSMK
VKHQVRLGISENSKATQEGLFQQFGLKESDGDLDPFQLSGGMLRRVLFTTCIS
DKVSLIIADEPTPGLHPDALQMVLDQLRSFADKGISVIFITHDIVAASQIADRITI
FKEGKAIETAPASFFSGNGEQLQTEFARSLWRSLPQQEFLKGVTHDLRG*

FIG. 1CONT'D

Sequence description

A) Length: 804 bp - 268 aa (full length gene)
B) Sequence Characteristics:
No obvious leader peptide sequence
Orf is preceded by a potential Shine-Dalgarno sequence.
This gene was not isolated using the LEEP system. However in determining a full length gene sequence for ID-76, this gene was identified downstream and fully sequenced.

ID-79

Clone 3-5c

GTCCATCTGGGGTGGTTCCCGATTGGTATTTCTTCTCCGATAGGTACTTTGA
GTCAAGATATTACGTTAGCTGATCGTATTAAGCACCTTATTTTACCTGTTTT
CACGGTAAGTATTCTAGGCATTGCCAATGTAACTCTTCATACTAGAACTAA
AATGATGTCGGTACTTTCTAGTGAATATGTCTTATTTGCCAGAGCGCGTGG
GGAAACGGAATGGCAAATTTTTAAAAATCATTGTCTTAGAAATGCTATCGT
ACCAGCTATTACACTGCATTTTTCCTATTTTGGAGAATTGTTTGGAGGATCC
GTTCTTGCTGAGCAAGTTTTCTCATATCCAGGACTAGGGTCTACCCTAACT
GAAGCAGGACTTAAAAGTGATACACCGCTACTTCTAGCTATTGTGATGATA
GGGACATTATTTGTTTTTGCGGGCAATCTTATTGCGGATATTTTAAATAGC
ATAATCAATCCACAGTTAAGGAGAAAAGTATGA

VHLGWFPIGISSPIGTLSQDITLADRIKHLILPVFTVSILGIANVTLHTRTKMMSV
LSSEYVLFARARGETEWQIFKNHCLRNAIVPAITLHFSYFGELFGGSVLAEQVF
SYPGLGSTLTEAGLKSDTPLLLAIVMIGTLFVFAGNLIADILNSIINPQLRRKV*

Sequence description

A) Length: 495 bp - 165 aa (partial gene sequence)
B) Sequence Characteristics:
N-terminus has yet to be determined.
This gene was not isolated using the LEEP system. However in determining a full length gene sequence for ID-76, this gene was identified upstream.

FIG. 1 CONT'D

ID-80

Clone 2-17

TTGCGGACAATTACGTTCAAACACAATGAAACGCGATCGTCAAAAAGCGA
AGGTAGGGCGGTAATGCTTAAAAGATTATTTACTGAAGATGGGGAATTGA
CAAAGATTAGTCGTCGTTTCGTTTGGATGTTAGTGGTTATCTATTGTCTTAT
TATTGTCAGGATGTGTTTTGGGCCTCAAATTATGATTGAGGGGGTATCAAC
TCCGAATGTTCAGCGCTTCGGAAGAATTGTAGCTCTTTTAGTACCATTTAA
TTCTTTTCGTAGTTTAGATCAGCTAACTAGCTTTAAAGAGATTTTTTGGGTT
ATTGGTCAAAATGTAGTGAATATTTTACTGCTGTTTCCTCTCATTATAGGGT
TACTATCCCTAAAGCCAAGTTTACGGAAATATAAAAGCGTTATATTACTTG
CTTTCTTGATGTCTCTTTTCATAGAGTGTACTCAAGTTGTTTTAGATATTTT
AATAGATGCTAATCGGGTTTTTGAAATCGACGATCTATGGACAAATACCTT
AGGCGGTCCTTTCGCCCTATGGAGTTATCGAAACATAAAAGGTTGGCTTCT
AACTATTAGAAAATGA

MRTITFKHNETRSSKSEGRAVMLKRLFTEDGELTKISRRFVWMLVVIYCLIIVR
MCFGPQIMIEGVSTPNVQRFGRIVALLVPFNSFRSLDQLTSFKEIFWVIGQNVV
NILLLFPLIIGLLSLKPSLRKYKSVILLAFLMSLFIECTQVVLDILIDANRVFEIDD
LWTNTLGGPFALWSYRNIKGWLLTIRK*

Sequence description

A) Length: 579 bp - 193 aa (full length gene)
B) Sequence Characteristics:
Possesses a potential leader peptide sequence
No obvious Shine-Dalgarno, but the 'TTG' codon
may not be the actual translation start point.
A methionine (ATG) that occurs ~22 codons
downstream of the 'TTG' is preceded by a
potential Shine-Dalgarno sequence and may
represent the actual start codon.

ID 81

Clone 3-1

FIG. 1CONT'D

TTGAAAAATTTAAATCGTTATGTAGTTGCGGTTTCTGGAGTCGTTTTACATT
TAATGCTAGGATCAACTTATGCTTGGAGTGTGTTTCGTAACCCAATTATCT
CAGAGACTGGTTGGGATATTTCATCAGTTTCATTCGCTTTTAGTTTGGCTAT
TTTTTGTCTAGGAATGTCTGCAGCTTTTATGGGACACTTAGTAGAGCGTTTT
GGTCCTAGGATAATGGGAATGATTTCTGCTATTTTATATGGAGCAGGGAAT
GTGTTAACAGGCTTAGCCATTGAAACTCAGCAGTTATGGTTACTGTATGTT
GCATACGGTATTTTAGGAGGAATCGGACTTGGTTCAGGTTATATTACTCCA
GTATCGACTATTATTAAATGGTTTCCTGATAGGAGGGGACTAGCAACAGG
ATTCGCTATTATGGGATTTGGCTTTGCTTCTTTAGTAACAAGTCCGCTTGCA
CAATCCTTACTGATTAGGATTGGTGTGGGTAAAACGTTTTATATTTTGGGA
TTAGTATATTTTTTTGTCATGATGATTGCCTCACAATTTATTAAACAACCAC
CTCAGGAAAAAATAACTATTTTGACTCACGATGGTAAAAAGAATGCTATG
AATTCACAAATTATCACTGGATTAAAAGCAAACGTCGCTATAAAATCAAA
AACCTTTTACATCATTTGGTTGACCTTGTTTATTAATATTTCGTGTGGCTTA
GGTTTAATATCAGCAGCTTCACCAATGGCACAAGATTTAGCAGGCTATTCC
GCAGAATCTGCAGCCTTATTAGTAGGGGTACTAGGGATATTTAACGGTTTT
GGACGTCTGTTATGGGCAAGTCTCTCTGACTACATTGGACGCCCGTTGACC
TTTATAATATTATTTATTGTGAACTTTATTATGACTTCTAGTTTATTTTTGTC
ATTCAATGCTATTGTATTTGCAATAGCGATGTCTATTTTAATGACTTGTTAT
GGTGCAGGTTTTTCCTTATTACCTGCTTATCTAAGTGATATTTTTGGAACAA
AGGAATTAGCTACTTTACATGGTTATAGTTTAACAGCATGGGCAATAGCAG
GTCTGTTTGGGCCCCTATTGTTATCAAAGACATATTCATGGGGAAATTCCT
ATCAATTGACATTAATGGTTTTTGGTTTTTTATTCTTATTCGGATTATTGTTA
TCTCTATATTTAAGAAAATTAACAACTAAAGTTGTGTAG

LKNLNRYVVAVSGVVLHLMLGSTYAWSVFRNPIISETGWDISSVSFAFSLAIFC
LGMSAAFMGHLVERFGPRIMGMISAILYGAGNVLTGLAIETQQLWLLYVAYG
ILGGIGLGSGYITPVSTIIKWFPDRRGLATGFAIMGFGFASLVTSPLAQSLLIRIG
VGKTFYILGLVYFFVMMIASQFIKQPPQEKITILTHDGKKNAMNSQIITGLKAN
VAIKSKTFYIIWLTLFINISCGLGLISAASPMAQDLAGYSAESAALLVGVLGIFN
GFGRLLWASLSDYIGRPLTFIILFIVNFIMTSSLFLSFNAIVFAIAMSILMTCYGA
GFSLLPAYLSDIFGTKELATLHGYSLTAWAIAGLFGPLLLSKTYSWGNSYQLTL
MVFGFLFLFGLLLSLYLRKLTTKVV*

Sequence description:

A] Length 1221 bp - 407 a.a (full length gene).
B] TTG start codon with Shine-Dalgarno sequence upstream. Obvious signal peptide, with hydropathy plot exhibiting many possible membrane spanning regions, indicating protein to be transmembrane.

FIG. 1 CONT'D

ID-82

Clone 48

ATGGCAGATAAAAACAGAACATTTAAACTTGTAGGTGCAGGATCTTCTAG
CACACAAGAAAAAATTGAAAAGCCTGCTCTTTCGTTTATGCAAGATGCGTG
GCGTCGCTTGAAAAAAAACAAATTAGCAGTAGTTTCACTCTATTTATTAGC
TCTTTTACTTACTTTTTCGTTAGCCTCAAATTTATTTGTAACTCAGAAGGAT
GCTAATGGGTTTGATTCGAAAAAAGTAACGACATATCGCAACTTACCACCT
AAATTGAGTTCAAACCTTCCTTTTTGGAATGGTAGCATTAATCCATCA

MADKNRTFKLVGAGSSSTQEKIEKPALSFMQDAWRRLKKNKLAVVSLYLLA
LLLTFSLASNLFVTQKDANGFDSKKVTTYRNLPPKLSSNLPFWNGSINPS

Sequence description:

A] Current length is 303 bp - 101 aa
B] No obvious signal peptide but Shine
Dalgarno sequence upstream of the ATG start
codon. Not ide3ntified directly using the LEEP system but was found directly downstream of ID-34 described in WO 00/06736.

ID-83

Clone 98

ATGAAAATAGTAGTACCAGTAATGCCTCGCAGTCTTGAAGAGGCTCAAGA
AATAGATTTATCAAAATTTGATAGTGTTGATATTATTGAATGGCGAGCTGA
TGCCTTACCAAAGGATGACATTATTAATGTAGCTCCAGCTATTTTTGAGAA
ATTCGCAGGTCATGAAATTATTTTTACTTTTCGTACAACGCGTGAAGGTGG
TAATATTGTCTTATCTGATGCTGAGTATGTTGAGTTAATCCAGAAAATTAA
TTCTATCTACAATCCAGATTATATTGATTTTGAGTATTTTTCACATAAAGAA
GTTTTTCAAGAAATGCTAGAATTTCCAAATTTAGTCCTGTCTTATCACAATT
TTCAAGAGACACCGGAGAATATTATGGAGATATTTTCAGAATTAACAGCC
CTAGCACCACGAGTTGTGAAAATCGCAGTAATGCCAAAGAATGAACAAGA

FIG. 1CONT'D

TGTCTTAGACGTTATGAATTACACTCGCGGTTTCAAGACTATTAATCCTGA
TCAAGTTTATGCGACGGTATCTATGAGTAAAATTGGACGTATTTCTCGTTTT
GCTGGTGATGTAACTGGATCTAGTTGGACATTTGCATATTTAGATTCATCT
ATCGCACCCGGACAAATTACTATTTCAGAGATGAAGCGTGTCAAAGCATT
GCTTGACGCTGACTGA

MKIVVPVMPRSLEEAQEIDLSKFDSVDIIEWRADALPKDDIINVAPAIFEKFAG
HEIIFTFRTTREGGNIVLSDAEYVELIQKINSIYNPDYIDFEYFSHKEVFQEMLEF
PNLVLSYHNFQETPENIMEIFSELTALAPRVVKIAVMPKNEQDVLDVMNYTRG
FKTINPDQVYATVSMSKIGRISRFAGDVTGSSWTFAYLDSSIAPGQITISEMKRV
KALLDAD*

Sequence description:

A] Length 678 bp, 225 aa (full length gene)
B] No obvious signal peptide, but there is a
Shine Dalgarno immediately upstream of ORF.

ID-84

Clone RS-52

ATGAAAGACTTATTTGCAACAACAGAAGCATCATCAAGGAAACAGGAACA
AGATAGAATTGTCAATTACATAAAACAACATGTTGAGTTAACAAATGGTA
ATCAAATAAAAAAAAATTGAGTTTATCGACTTTCAAAAAAATGAGATGACA
GGTACATGGGGAATTTCTACTAAAATTAATGAACAATTTTCGATTAGTTTT
TCTGAAGATAGAATTGGTGGTAAACTTAGAGCATTAGGATATCAACCGAA
TGAAATAGGTTTTTCAAAGGACATCAATAGTAATAATCAAAATGTTAATGA
TATTGAAGTGATTTATATGAAGAAAGAATAG

MKDLFATTEASSRKQEQDRIVNYIKQHVELTNGNQIKKIEFIDFQKNEMTGTW
GISTKINEQFSISFSEDRIGGKLRALGYQPNEIGFSKDINSNNQNVNDIEVIYMK
KE*

Sequence description:

A] length: 333 bp - 111 aa (partial sequence)
B] No obvious Shine Dalgarno sequence upstream
of the ATG start codon, and no obvious signal
peptide within the protein.

FIG. 1CONT'D

ID-85

Clone RS-53

ATGAAAAAACGTATATGGTATTTGATAATAATAATCACAGTAATTTTAGGA
GGACTAGCCATGAAAAACTTATTTGCAACAACAGAAGCATCATCAAGGAA
ACAGGAACAAGATAGAATTGTCAATTACATAAAACAACATGTTGAGTTAA
CAAATGGTAATCAAATAAAAAAAAATTGAGTTTATCGACTTTCAAAAAAAT
GAGATGACAGGTACATGGGGAATTTCTACTAAAATTAATGAACAATTTTCG
ATTAGTTTTTCTGAAGATAGAATTGGTGGTAAACTTAGAGCATTAGGATAT
CAACCGAATGAAATAGGTTTTTCAAAGGACATCAATAGTAATAA**TCA**

MKKRIWYLIIIITVILGGLAMKNLFATTEASSRKQEQDRIVNYIKQHVELTNGN
QIKKIEFIDFQKNEMTGTWGISTKINEQFSISFSEDRIGGKLRALGYQPNEIGFSK
DINSNNQ

Sequence description:

A] Length: 351 bp - 117 aa (Partial sequence)
B] Obvious signal peptide and Shine Dalgarno sequence upstream of the ATG start codon.

ID-86

Clone ID-74

ATGTCAAATCAATATGATTATATCGTTATTGGTGGAGGTAGT
GCAGGCAGTGGTACCGCTAATAGGGCAGCCATGTATGGAGC
AAAAGTCCTGTTAATTGAAGGTGGACAAGTAGGTGGAACTTG
TGTTAACTTAGGTTGTGTACCTAAGAAAATCATGTGGTATGG
TGCACAAGTTTCTGAGACACTCCATAAGTATAGTTCAGGTTA
TGGTTTTGAAGCCAATAATCTTAGTTTTGATTTTACTACTCTA
AAAGCTAATCGCGATGCTTACGTGCAGCGGTCTAGACAGTCG
TATGCCGCTAATTTTGAGCGTAATGGGGTCGAAAAGATTGAT
GGATTTGCTCGTTTTATTGATAACCATACTATTGAAGTGAATG
GTCAGCAATATAAAGCTCCTCACATTACTATTGCAACAGGTG

FIG. 1CONT'D

GACACCCTCTTTACCCTGATATTATTGGAAGTGAACTTGGTG
AGACTTCTGATGATTTTTTTGGATGGGAGACCTTACCAAATTC
TATATTGATTGTTGGGGCGGGCTATATCGCGGCAGAACTTGC
TGGAGTGGTTAATGAATTAGGCGTTGAAACCCATCTTGCATT
TAGAAAAGACCATATTCTACGCGGATTTGATGACATGGTAAC
AAGTGAGGTTATGGCTGAAATGGAGAAATCAGGTATCTCTTT
ACATGCTAACCATGTACCTAAATCTCTTAAACGCGATGAAGG
TGGCAAGTTGATTTTTGAAGCTGAAAATGGGAAAACGCTTGT
CGTTGATCGTGTAATATGGGCTATCGGCCGTGGACCAAATGT
AGACATGGGACTTGAAAATACCGATATTGTTTTAAATGATAA
AGATTATATCAAAACAGATGAATTTGAGAATACTTCTGTAGA
TGGCGTGTATGCTATTGGAGATGTTAATGGGAAAATTGCCTT
GACACCGGTAGCAATTGCAGCAGGTCGTCGCTTATCAGAAAG
ACTTTTTAATCATAAAGATAACGAAAAATTAGATTACCATAA
TGTACCTTCAGTTATTTTTACTCACCCTGTAATTGGGACGGTA
GGACTTTCAGAAGCAGCAGCTATCGAGCAATTTGGAAAAGAT
AATATCAAAGTCTATACATCAACTTTTACCTCTATGTATACGG
CTGTTACCAGTAATCGCCAAGCAGTTAAGATGAAGCTCATAA
CCCTAGGAAAAGAGGAAAAAGTTATTGGGCTTCATGGTGTTG
GTTATGGTATTGATGAAATGATTCAAGGTTTTTCAGTTGCTAT
CAAAATGGGGGCTACTAAAGCAGACTTTGATGATACTGTTGC
TATTCACCCAACTGGATCTGAGGAATTTGTTACAATGCGCTA
A

MSNQYDYIVIGGGSAGSGTANRAAMYGAKVLLIEGGQVGGTC
VNLGCVPKKIMWYGAQVSETLHKYSSGYGFEANNLSFDFTTLK
ANRDAYVQRSRQSYAANFERNGVEKIDGFARFIDNHTIEVNGQ
QYKAPHITIATGGHPLYPDIIGSELGETSDDFFGWETLPNSILIVG
AGYIAAELAGVVNELGVETHLAFRKDHILRGFDDMVTSEVMAE
MEKSGISLHANHVPKSLKRDEGGKLIFEAENGKTLVVDRVIWAI
GRGPNVDMGLENTDIVLNDKDYIKTDEFENTSVDGVYAIGDVN
GKIALTPVAIAAGRRLSERLFNHKDNEKLDYHNVPSVIFTHPVIG
TVGLSEAAAIEQFGKDNIKVYTSTFTSMYTAVTSNRQAVKMKLI
TLGKEEKVIGLHGVGYGIDEMIQGFSVAIKMGATKADFDDTVAI
HPTGSEEFVTMR*

ID-87

FIG. 1 CONT'D

Clone RS-55

ATGACAAAAAAACATCTTAAAACGCTTGCCTTGGCACTTACTACAGTATCA
GTAGTGACATACAGCCAGGAGGTATATGGATTAGAAAGAGAGGAATCGGT
CAAACAAGAACAAACCCAGTCAGCTTCAGAAGATGATTGGTTCGAAGAAG
ATAATGAGAGGAAAACAAATGTTTCTAAAGAGAATTCTACTGTTGATGAA
ACAGTTAGTGATTTATTTTCTGATGGAAATAGTAATAACTCTAGTTCTAAA
ACCGAGTCAGTGGTAAGTGACCCTAAACAAGTCCCCAAAGCAAAACCAGA
GGTTACACAAGAAGCAAGCAATTCTAGTAATGATGCTAGCAAAGTAGAAG
TACCAAAACAGGATACAGCTTCAAAAAAGGAAACTCTAGAAACATCAACT
TGGGAGGCAAAAGATTTCGTAACTAGAGGGGATACTTTAGTAGGTTTTTCA
AAATCTGGAATTAATAAGTTATCTCAAACATCACACTTGGTTTTACCAAGT
CATGCAGCAGATGGAACTCAATTGACACAAGTAGCTAGCTTTGCTTTTACT
CCAGATAAAAAAGACGGCCATTGCAGAATATACAAGTAGGCTAGGAGAAA
ATGGGAAACCGAGTCGTTTAGATATTGATCAGAAGGAAATTATTGATGAG
GGAGAAATATTTAATGCTTACCAGTTGACTAAGCTTACTATTCCAAATGGT
TATAAGTCTATTGGTCAAGATGCTTTTGTGGACAATAAGAATATTGCTGAG
GTTAACCTTCCTGAGAGTCTCGAGACTATTTCAGACTATGCTTTTGCTCACA
TGTCTTTAAAACAAGTAAAGTTACCAGATAACCTAAAGGTCATTGGAGAA
TTAGCTTTTTTTGATAATCAGATTGGTGGTAAGCTTTACTTGCCACGTCACT
TGATAAAATTAGCAGAACGCGCTTTCAAATCTAATCGTATTCAAACAGTTG
AATTTTTGGGAAGTAAGCTTAAGGTTATAGGAGAAGCAAGTTTTCAAGAT
AATAATCTGAGGAATGTTATGCTTCCGGATGGACTTGAAAAAATAGAATC
AGAAGCTTTTACAGGAAATCCAGGAGATGAACATTACAACAATCAGGTTG
TATTGCGCACAAGGACAGGCCAAAATCCACATCAACTTGCGACTGAGAAT
ACTTACGTCAATCCGGACAAATCATTGTGGCGTGCAACACCTGATATGGAT
TATACCAAATGGTTAGAGGAAGATTTTACCTATCAAAAAAATAGTGTTACA
GGTTTTTCAAATAAAGGCTTACAAAAGGTAAGACGTAATAAAAACTTAGA
AATTCCAAAACAACACAATGGTATTACTATTACTGAAATTGGTGATAACGC
TTTTCGCAATGTTGATTTTCAAAGTAAAACTTTACGTAAATATGATTTGGA
AGAAATAAAGCTCCCCTCAACTATTCGGAAAATAGGTGCTTTTGCTTTTCA
ATCTAATAACTTGAAATCCTTTGAAGCAAGTGAAGATTTAGAAGAGATTA
AAGAGGGAGCCTTTATGAATAATCGTATTGGAACTCTAGACTTGAAAGAC
AAACTTATCAAAATAGGTGATGCTGCTTTCCATATTAATCATATTTATGCC
ATTGTTCTTCCAGAATCTGTACAAGAAATAGGACGTTCAGCTTTTCGACAA
AATGGTGCGCTTCACCTTATGTTTATCGGAAATAAGGTTAAAACAATTGGT
GAAATGGCTTTTTTATCCAATAAACTGGAAAGTGTAAATCTCTCTGAGCAA
AAACAATTAAAGACAATTGAGGTCCAAGCTTTTTCGGATAATGCCCTTAGT
GAAGTAGTCTTACCGCCAAATTTACAGACTATTCGTGAAGAGGCTTTCAAA
AGGAATCATTTGAAAGAAGTGAAGGGTTCATCTACATTATCTCAGATTACT
TTTAATGCTTTTGATCAAAATGATGGGGACAAACGCTTTGGTAAGAAAGTG
GTTGTTAGGACACATAATAATTCTCATATGTTAGCAGATGGTGAGCGTTTT
ATCATTGATCCAGATAAGCTATCTTCTACAATGGTAGACCTTGAAAAGGTT

FIG. 1CONT'D

TTAAAAATAATCGAAGGTTTAGATTACTCTACATTACGTCAGACTACTCAA
ACTCAGTTTAGAGAAATGACTACTGCAGGTAAAGCGTTGTTATCAAAATCT
AACCTCCGACAAGGAGAAAAACAAAAATTCCTTCAAGAAGCACAATTTTT
CCTTGGTCGCGTTGATTTGGATAAAGCCATAGCTAAAGCTGAGAAGGCTTT
AGTGACCAAGAAGGCAACAAAGAATGGTCATTTGCTTGAGAGGAGTATTA
ACAAAGCGGTATTAGCTTATAATAATAGTGCTATTAAAAAAGCTAATGTTA
AGCGCTTGGAAAAAGAGTTAGACTTGCTGACAGATTTAGTCGAGGGAAAA
GGACCATTAGCGCAAGCTACAATGGTACAAGGAGTTTATTTATTAAAGAC
GCCTTTACCATTGCCAGAATATTATATCGGATTGAACGTTTATTTTGACAA
GTCTGGAAAATTGATTTATGCACTTGATATGAGTGATACTATTGGCGAGGG
ACAAAAAAGATGCATATGGTAATCCTATATTAAATGTTGACGAGGATAATG
AAGGTTATCATACCTTGGCAGTTGCCACTTTAGCTGATTATGAAGGTCTTT
ATATTAAAGATATTTTAAATAGTTCCCTTGATAAGATTAAAGCAATACGCC
AGATTCCTTTGGCAAAATATCATAGATTAGGAATTTTCCAAGCTATCCGAA
ATGCAGCGGCAGAAGCAGACCGATTGCTTCCTAAGACACCTAAGGGGTAC
CTAAATGAAGTCCCAAATTATCGTAAAAAACAAATGGAGAAAAATTTAAA
ACCAGTTGATTATAAAACGCCGATTTTTAATAAGGCTTTACCTAATGAAAA
GGTAGACGGTGATAGAGCGGCTAAAGGTCATAATATAAATGCGGAGACTA
ATAATTCTGTAGCTGTAACACCAATAAGGTCCGAGCAGCAATTACATAAGT
CACAGTCTGATGTAAATTTACCTCAAACAAGTTCTAAAAATAATTTTATAT
ACGAGATTCTAGGATACGTTAGTTTATGTTTGCTTTTCCTAGTAACTGCTGG
GAAAAAAGGAAAACGAGCAAGAAAATAA

MTKKHLKTLALALTTVSVVTYSQEVYGLEREESVKQEQTQSASEDDWFEEDN
ERKTNVSKENSTVDETVSDLFSDGNSNNSSSKTESVVSDPKQVPKAKPEVTQE
ASNSSNDASKVEVPKQDTASKKETLETSTWEAKDFVTRGDTLVGFSKSGINKL
SQTSHLVLPSHAADGTQLTQVASFAFTPDKKTAIAEYTSRLGENGKPSRLDIDQ
KEIIDEGEIFNAYQLTKLTIPNGYKSIGQDAFVDNKNIAEVNLPESLETISDYAF
AHMSLKQVKLPDNLKVIGELAFFDNQIGGKLYLPRHLIKLAERAFKSNRIQTV
EFLGSKLKVIGEASFQDNNLRNVMLPDGLEKIESEAFTGNPGDEHYNNQVVLR
TRTGQNPHQLATENTYVNPDKSLWRATPDMDYTKWLEEDFTYQKNSVTGFS
NKGLQKVRRNKNLEIPKQHNGITITEIGDNAFRNVDFQSKTLRKYDLEEIKLPS
TIRKIGAFAFQSNNLKSFEASEDLEEIKEGAFMNNRIGTLDLKDKLIKIGDAAFH
INHIYAIVLPESVQEIGRSAFRQNGALHLMFIGNKVKTIGEMAFLSNKLESVNL
SEQKQLKTIEVQAFSDNALSEVVLPPNLQTIREEAFKRNHLKEVKGSSTLSQITF
NAFDQNDGDKRFGKKVVVRTHNNSHMLADGERFIIDPDKLSSTMVDLEKVL
KIIEGLDYSTLRQTTQTQFREMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGR
VDLDKAIAKAEKALVTKKATKNGHLLERSINKAVLAYNNSAIKKANVKRLEK
ELDLLTDLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA
LDMSDTIGEGQKDAYGNPILNVDEDNEGYHTLAVATLADYEGLYIKDILNSSL
DKIKAIRQIPLAKYHRLGIFQAIRNAAAEADRLLPKTPKGYLNEVPNYRKKQM
EKNLKPVDYKTPIFNKALPNEKVDGDRAAKGHNINAETNNSVAVTPIRSEQQL
HKSQSDVNLPQTSSKNNFIYEILGYVSLCLLFLVTAGKKGKRARK*

FIG. 1 CONT'D

Sequence description:

A] Length 3168 bp - 1056 aa (Partial sequence)
B] Obvious signal peptide with Shine Dalgarno sequence upstream of the ATG start codon.

ID-88

Clone RS-56

GCAGGATACATCATGCACAAGCACGAGGCTATCGTGTCATGCTGGGGTCA
ACCCAGGAAGACATGTCGGCACAAGCTGAAGATTTCTTTACAGTCTGTACA
CAATAAAGAGACGGGTAAGAGCGCTTTTAATGACAAAGAACGACTAGCAA
TT

AGYIMHKHEAIVSCWGQPRKTCRHKLKISLQSVHNKETGKSAFNDKERLAI

Sequence description:

A] Length:153 bp - 51 aa (partial sequence)
B] No signal peptide visible, insufficient sequence data to determine the presence of a Shine Dalgarno sequence.

ID-89

Clone RS-58

GTGTCATTTATGCAAAGAAAATCCTATTTAAAATCCATGAGTGTTCTTACT
TTAACAGCTTGTCTTATATCAGGATATGTGGTTAAAGATATTGCTATGTTA
CATGCAGTATCTGCCAGTGAGAAGAAAGCAAATAATGTCAGTCCGAGAGA
AAATCTCTACAGGGCTGTCAATGATAATTGGCTAGCCAATACAAAACTCA
AACAAGGGCAGACTAGTGTTAATAGTTTTTCAGAAATTGAGGATAAATTA
AAGCAACTGTTAGTGTCTGATATGGCTAAAATGGCCTCAGGAAAGATTGA

FIG. 1CONT'D

AACAACCAATGATGAACAGAAAAAAATGGTTGCATACTATAAACAAGGTA
TGGACTTTAAAACAAGAGATAAAAATGGTCTCAAACCTCTAAAACCAGTT
TTACAAAAACTTGAAGCAGTCTCTTCAATGAAAGACTTTCAAAGTTTGGCC
CATGATTTTGTGATGAGTGGTTTTGTTTTACCATTTGGTTTGACTGTGGAAA
CCAATGCTCGAGATAATAGCCAAAAGCAATTGGTGCTTCGTCAAGCACCC
GCATTACTTGAATCACCTGACCAATATAAGAAGGGCAATAAAGAAGGTGA
GGCTAAATTATCAGCTTACCGTACTTCAGCAATGGCTTTGCTTAAACAAGC
TGGAAAAAGTAACATTGAAGATAGAAAACTAGTTAAACAAGCTATAGCAT
TTGATAGACTCTTATCAGAAAAAACGCAAGTTGATCAAAGTAAAATCACA
GCTGAAAGTGAGACAGCTGCGGGGCGATATAACCCTGAAAGTATGGAAAC
GGTTCACAATTACGCCAAGGAATTTGACTTTAAAGAATTGATTGAAAAACT
AGTTGGGCCAACGAATAAGGCAGTCAATGTAGAAGATAAAACTTATTTTA
AACAGGTTAATGATGTTATAAATAGTAAACAATTAGCCAATATGAAAGCA
TGGATGATGATTTCTATGCTAGTTGATCAATCAGATTTTCTAGGAGAACAA
AATCGTCAAGCAGCGAGTGCTTTTAAGAATGTTGCGTCTGGTTTGACTCAG
ATTGAATCGAAAGAAAAAATGCTTACACCCAATTAG

MSFMQRKSYLKSMSVLTLTACLISGYVVKDIAMLHAVSASEKKANNVSPREN
LYRAVNDNWLANTKLKQGQTSVNSFSEIEDKLKQLLVSDMAKMASGKIETTN
DEQKKMVAYYKQGMDFKTRDKNGLKPLKPVLQKLEAVSSMKDFQSLAHDF
VMSGFVLPFGLTVETNARDNSQKQLVLRQAPALLESPDQYKKGNKEGEAKLS
AYRTSAMALLKQAGKSNIEDRKLVKQAIAFDRLLSEKTQVDQSKITAESETAA
GRYNPESMETVHNYAKEFDFKELIEKLVGPTNKAVNVEDKTYFKQVNDVINS
KQLANMKAWMMISMLVDQSDFLGEQNRQAASAFKNVASGLTQIESKEKMLT
PN*

Sequence description:

A] Length: 1095 bp - 365 aa (full length gene)
B] an GTG (possible ATG start codon located 7 bp further downstream) start codon with an obvious signal peptide. Shine Dalgarno sequence present upstream of the ORF.

ID-90

Clone RS-59

FIG. 1CONT'D

ATGGAAATGCCTAAAAGAAATGAATTACTCAATAAAGAAATTAAAATGAG
TATTGATAAACTTAGATATAAAGAACCAGAGAGTGAACATGACAAGCGAC
CTACTTTTTATTTGGTAGTACTTATACTTGTTACTGTAGCAGTTATATTGTC
GTTATTTAAATATTTTTTATAG

MEMPKRNELLNKEIKMSIDKLRYKEPESEHDKRPTFYLVVLILVTVAVILSLFK
YFL*

Sequence description:

A] Length: 174 bp - 58 aa(full length gene)
B] No obvious signal peptide, but Shine Dalgarno sequence is present upstream of ATG start codon.

ID-91

Clone RS-62 (partial sequence)

ATGCAGGTATTTTTAAATATTGTCAATAAATTCTTTGATCCAGTTATTCATA
TGGGTTCGGGAGTTGTGATGCTAATTGTCATGACAGGTTTAGCCATGATAT
TTGGAGTGAAGTTTTCTAAAGCACTTGAAGGTGGTAT

MQVFLNIVNKFFDPVIHMGSGVVMLIVMTGLAMIFGVKFSKALEGG

Sequence description:

A] Length:141 bp - 41 aa (partial sequence
B] Shine Dalgarno sequence present upstream of ATG start codon with a possible signal peptide present

ID-92

FIG. 1 CONT'D

Clone RS-69 (partial sequence)

ATGAAAAAGAAAACATTCAGTGCTTATAACTTTTTAACGGCTCTTATCCTT
TGTCTTTTGACAGTGCTTTTTATCTTTCCATTTTATTGGATTATGACAGGAG
CTTTTAA

MKKKTFSAYNFLTALILCLLTVLFIFPFYWIMTGAF

Sequence description:

A] Length: 110 bp -36 aa (Partial sequence)
B] Possible signal peptide with Shine Dalgarno sequence directly upstream of the ATG start codon.

ID-93

Clone RS-70

ATGACTGAGAACTGGTTACATACTAAAGATGGTTCAGATATTTATTATCGT
GTCGTTGGTCAAGGTCAACCGATTGTTTTTTTACATGGCAATAGCTTAAGT
AGTCGCTATTTTGATAAGCAAATAGCATATTTTTCTAAGTATTACCAAGTT
ATTGTTATGGATAGTAGAGGGCATGGCAAAAGTCATGCAAAGCTAAATAC
CATTAGTTTCAGGCAAATAGCAGTTGACTTAAAGGATATCTTAGTTCATTT
AGAGATTGATAAAGTTATATTGGTAGGCCATAGCGATGGTGCTAATTTAGC
TTTAGTTTTTCAAACGATGTTTCCAGATATGGTTAGAGGGCTTTTGCTTAAT
TCAGGGAACCTGACTATTCATGGTCAGCGATGGTGGGATATTCTTTTAGTA
AGGATTGCCTATAAATTCCTTCACTATTTAGGGAAACTCTTTCCGTATATG
AGGCAAAAAGCTCAAGTTATTTCGCTTATGTTGGAGGATTTGAAGATTAGT
CCAGCTGATTTACAGCATGTGTCAACTCCTGTAATGGTTTTGGTTGGAAAT
AAGGACATAATTAAGTTAAATCATTCTAAGAAACTTGCTTCTTATTTTCCA
AGGGGGGAGTTTTATTCTTTAGTTGGCTTTGGGCATCACATTATTAAGCAA
GATTCCCATGTTTTTAATATTATTGCAAAAAAGTTTATCAACGATACGTTG
AAAGGAGAAATTGTTGAAAAAGCTAATTGA

MTENWLHTKDGSDIYYRVVGQGQPIVFLHGNSLSSRYFDKQIAYFSKYYQVIV
MDSRGHGKSHAKLNTISFRQIAVDLKDILVHLEIDKVILVGHSDGANLALVFQ

FIG. 1 CONT'D

TMFPDMVRGLLLNSGNLTIHGQRWWDILLVRIAYKFLHYLGKLFPYMRQKA
QVISLMLEDLKISPADLQHVSTPVMVLVGNKDIIKLNHSKKLASYFPRGEFYSL
VGFGHHIIKQDSHVFNIIAKKFINDTLKGEIVEKAN*

Sequence description:

A] Length: 744 bp - 248 aa (full length gene)
B] No obvious signal peptide, but Shine Dalgarno sequence upstream of the ATG start codon.

ID-94

Clone RS-71

ATGGTAGCAAAAGAGTTAGGTAAAAATAGCTTTACTATCCCAACTATTTGT
TCTAATTGCTCCGCAGGTACTGCCATTGCAGTTGTATATAATGATGACCAT
TCTTTCTTAAGATACGGCTATCCCGAGTCTCCACTTCATATTTTTATCAATA
CACGGATCATTGCACAGGCACCAAGCAAATATTTTTGGGCTGGTATTGGGG
ACGGTATTTCAAAAGCCCCTGAAGTAGAACGTGCTACCTTAGAGGCTAAG
ACCAATAAACTACCACATACTGCAGTGTTAGGACAAGCAGTCGCTCTGTCT
TCAAAGGAAGCTTTTTATCAATTTGGTGAACAAGGTCTAAAAGACGTTGAA
GCTAATTTAGCTTCGCGTGCAGTTGAAGAAATTGCGCTTGATATCTTA

MVAKELGKNSFTIPTICSNCSAGTAIAVVYNDDHSFLRYGYPESPLHIFINTRIIA
QAPSKYFWAGIGDGISKAPEVERATLEAKTNKLPHTAVLGQAVALSSKEAFY
QFGEQGLKDVEANLASRAVEEIALDIL

Sequence description:

A] Length: 405 bp - 135 aa (Partial sequence)
B] No obvious Shine Dalgarno sequence upstream of the ATG start codon, probable signal peptide present at the N-terminus.

ID-95

FIG. 1CONT'D

Clone RS-73

TTGAGGGAAACTTACTGGAAAATTTCAAGCGATTGCGATAAAATAAATCTT
GCAGAGTTTTCTAGAGAAAGGAGGTCAGATTTATTGGAGTGGCAAGATCT
AGCGCAGTTACCTGTATCTATTTTTAAAGACTATGTTACAGATGCTCAAGA
CGCGGAAAAACCTTTTATATGGACAGAAGTATTTTTAAGGGAGATTAATCG
CTCAAATCAAGAAATTATTTTGCATATTTGGCCGATGACTAAGACAGTCAT
TCTGGGGATGTTAGATCGAGAATTACCACATTTAGAATTAGCTAAAAAAG
AAATCATCAGTCGTGGTTATGAACCAGTTGTTCGGAATTTTGGAGGTCTCG
CAGTTGTAGCTGATGAAGGAATTTTAAATTTTTCATTGGTTATTCCAGATGT
TTTTGAGAGAAAATTGTCTATCTCAGATGGGTATCTTATAATGGTCGATTTT
ATTAGAAGTATATTTTCGGATTTTTATCAACCTATTGAGCACTTTGAAGTA
GAGACCTCCTATTGTCCTGGTAAGTTTGATCTTAGTATAAATGGCAAAAAA
TTTGCTGGCTTGGCTCAGCGCCGTATAAAGAATGGTATTGCGGTATCAATT
TACCTTAGCGTTTGTGGCGATCAAAAAGGGCGGAGTCAAATGATTTCAGAT
TTTTATAAGATTGGTCTAGGTGATACGGGTAGTCCAATTGCTTATCCAAAT
GTAGATCCTGAAATTATGGCTAATCTATCTGATCTATTAGATTGTCCTATG
ACAGTAGAAGATGTTATTGATCGTATGTTGATTAGCCTTAAACAAGTAGGT
TTTAATGATCGTTTACTGATGATTAGACCCGATTTAGTTGCAGAGTTTGAT
AGATTTCAGGCTAAGTCTATGGCTAATAAGGGGATGGTGAGCAGAGATGA
ATAA

MRETYWKISSDCDKINLAEFSRERRSDLLEWQDLAQLPVSIFKDYVTDAQDAE
KPFIWTEVFLREINRSNQEIILHIWPMTKTVILGMLDRELPHLELAKKEIISRGYE
PVVRNFGGLAVVADEGILNFSLVIPDVFERKLSISDGYLIMVDFIRSIFSDFYQPI
EHFEVETSYCPGKFDLSINGKKFAGLAQRRIKNGIAVSIYLSVCGDQKGRSQMI
SDFYKIGLGDTGSPIAYPNVDPEIMANLSDLLDCPMTVEDVIDRMLISLKQVGF
NDRLLMIRPDLVAEFDRFQAKSMANKGMVSRDE*

Sequence description:

A] Length: 921 bp -307 aa (Full-length gene sequence)
B] No obvious Shine Dalgarno sequence upstream of the TTG start codon or signal peptide visible. Actual start point may be a further 85 bp downstream (TTG). This start point is preceded by a typical Shine-Dalgarno sequence.

FIG. 1 CONT'D

ID-96

Clone RS-74

TTGGAAGGTTTACTTATTGCATTGATTCCCATGTTTGCGTGGGAAAGTATT
GGATTTGTTAGTAATAAAATTGGAGGGCGTCCAAATCAACAAACATTTGG
AATGACTTTAGGAGCATTGCTATTTGCGATTATCGTATGGTTATTTAAACA
GCCAGAGATGACTGCCTCATTGTGGATTTTTGGTATCTTAGGTGGTATCCT
ATGGTCAGTCGGCCAAAATGGTCAATTTCAAGCAATGAAATATATGGGAG
TCTCTGTTGCTAATCCACTGTCAAGTGGTGCACAATTAGTAGGTGGAAGCC
TAGTTGGTGCTTTAGTCTTTCATGAATGGACTAAGCCAATCCAATTTATTTT
AGGATTGACAGCGTTGACATTATTAGTTATCGGCTTCTATTTCTCAAGTAA
ACGTGATGTTTCAGAACAAGCTTTGGCAACACATCAAGAGTTTTCAAAAG
GATTTGCTACAATTGCTTATTCAACTGTAGGTTACATCTCGTACGCAGTTTT
ATTTAACAACATTATGAAGTTCGACGCTATGGCCGTCATTTTACCCATGGC
TGTTGGAATGTGTCTAGGTGCAATTTGTTTCATGAAGTTTCGTGTTAACTTT
GAGGCTGTTGTTGTTAAAAATATGATTACAGGTCTCATGTGGGGCGTTGGT
AATGTCTTCATGTTATTGGCAGCAGCTAAAGCAGGGCTAGCAATTGCTTTT
AGTTTTTCTCAACTTGGAGTAATTATCTCTATTATTGGTGGTATTTTATTTTT
AGGTGAGACAAAAACGAAGAAAGAGCAGAAATGGGTTGTCATGGGTATC
CTTTGTTTTGTTATGGGTGCTATATTACTTGGTATTGTTAAATCTTATTAA

MEGLLIALIPMFAWESIGFVSNKIGGRPNQQTFGMTLGALLFAIIVWLFKQPEM
TASLWIFGILGGILWSVGQNGQFQAMKYMGVSVANPLSSGAQLVGGSLVGAL
VFHEWTKPIQFILGLTALTLLVIGFYFSSKRDVSEQALATHQEFSKGFATIAYST
VGYISYAVLFNNIMKFDAMAVILPMAVGMCLGAICFMKFRVNFEAVVVKNMI
TGLMWGVGNVFMLLAAAKAGLAIAFSFSQLGVIISIIGGILFLGETKTKKEQK
WVVMGILCFVMGAILLGIVKSY*

Sequence description:

A] Length: 867 bp - 289 aa (full-length gene)
B] Posible Shine Dalgarno sequence upstream of GTG start codon, no obvious signal peptide present.

ID-97

FIG. 1 CONT'D

Clone RS-75

ATGACAACTTACTACGAAGCTATAAACTGGAACGAAATTGAAGATGTTAT
TGATAAATCAACTTGGGAAAAACTAACCGAACAATTTTGGCTCGATACAC
GTATCCCTTTATCAAATGACTTAGACGATTGGCGCAAACTTTCCGCTCAAG
AAAAAGATCTTGTTGGCAAGGTTTTTGGAGGCTTAACCCTACTTGATACCA
TGCAATCAGAAACTGGTGTTGAAGCTATTCGTGCCGATGTTCGCACGCCTC
ACGAAGAAGCTGTCTTAAACAATATTCAATTCATGGAATCTGTTCACGCTA
AATCTTATTCTTCAATTTTCTCAACTTTAAATACTAAATCAGAAATTGAAG
AAATTTTCGAGTGGACTAATAATAATGAGTTCCTTCAAGAAAAAAGCACGT
ATTATCAATGACATTTATGCTAATGGAAATGCCCTTCAAAAAAAAGGTGGCT
TCCACCTACCTCGAAACTTTCCTTTTTTATTCTGGCTTTTTCACACCTCTTTA
CTATTTGGGAAATAATAAGTTAGCAAATGTTGCTGAAATCATTAAATTAAT
TATTCGTGATGAATCTGTACATGGTACTTATATCGGTTACAAATTCCAGCTT
GGTTTTAACGAATTACCAGAAGATGAGCAAGAGAATTTTCGTGATTGGAT
GTATGACCTCCTTTATCAGCTGTATGAAAACGAAGAAAAATACACCAAGA
CACTTTATGATGGCGTAGGATGGACTGAAGAAGTTATGACCTTTTTACGCT
ACAATGCTAATAAAGCTCTTATGAATTTAGGACAAGATCCTTTATTCCCAG
ATACAGCAAATGATGTCAACCCAATTGTTATGAATGGTATTTCAACAGGAA
CATCAAACCATGACTTCTTCTCTCAAGTAGGTAATGGTTACCTACTTGGTA
GCGTTGAAGCTATGCATGATGATGACTATAACTATGGATTATAA

MTTYYEAINWNEIEDVIDKSTWEKLTEQFWLDTRIPLSNDLDDWRKLSAQEK
DLVGKVFGGLTLLDTMQSETGVEAIRADVRTPHEEAVLNNIQFMESVHAKSY
SSIFSTLNTKSEIEEIFEWTNNNEFLQEKARIINDIYANGNALQKKVASTYLETF
LFYSGFFTPLYYLGNNKLANVAEIIKLIIRDESVHGTYIGYKFQLGFNELPEDEQ
ENFRDWMYDLLYQLYENEEKYTKTLYDGVGWTEEVMTFLRYNANKALMNL
GQDPLFPDTANDVNPIVMNGISTGTSNHDFFSQVGNGYLLGSVEAMHDDDYN
YGL*

Sequence description:

A] Length: 960 bp - 320 aa (full length gene)
B] Shine Dalgarno sequence present upstream of ATG start codon, but no signal peptide present.

ID-98

FIG. 1CONT'D

Clone RS-77 (partial sequence)

ATGAATTGGTCACGTATCTGGGAACTCGTAAAAATTAATATCCTTTATTCA
AACCCTCAGACTCTATCGGCACTAAGAAAAAAGCAAGAAAAGCATCCTAA
AAAAGAATTTTCAGCTTATAAATCCATGTTTAGAAATCAGTTATTTCAGAT
TTTGCTCTTTTCAATAATTTATGTATTTCTCTTTGTATCACTTGATTTTAAAG
AATATCCGGGCTATTTCACGTTCTACATTGGTATCTTTACACTAGTATCCAT
TATCTACTCTTTTATTGCGATGTACAGTGTTTTCTATGAGAGTGACGATGTT
AA

MNWSRIWELVKINILYSNPQTLSALRKKQEKHPKKEFSAYKSMFRNQLFQILL
FSIIYVFLFVSLDFKEYPGYFTFYIGIFTLVSIIYSFIAMYSVFYESDDV

Sequence description:

A] Length: 311 bp - 103 aa (Partial sequence)
B] Shine Dalgarno sequence present upstream of ATG start codon, no obvious signal peptide at N-terminus.

ID-99

Clone RS-78 (partial sequence)

TAATCTTTTAGTCAACGGAGCAACAGGAAAATTGCAGGCTATGCGACAGA
TATTCCACCACATAATTTAGCAGAAGTCATTGATGCTGTCGTGTACATGAT
TGATCACCCTAAAGCTAAATTAGATAAATTAATGGAATTTCTACCTGGTCC
AGATTTTCCAACTGGCGCTATCATTCAAGGAAAAGATGAAATTCGTAAGG
CATATGAGACTGGTAAGGGGAGAGTAGCGGTTCGCTCGCGAACTGCTATT
GAAACCTTAAAAGGTGGTAAGAAACAAATTATTGTTACTGAAATTCCTTAT
GAAGTTAAT

SFSQRSNRKIAGYATDIPPHNLAEVIDAVVYMIDHPKAKLDKLMEFLPGPDFPT
GAIIQGKDEIRKAYETGKGRVAVRSRTAIETLKGGKKQIIVTEIPYEVN

Sequence description:

A] Length: 312 bp - 104 aa (Partial sequence)
B] No obvious Shine Dalgarno sequence or a

FIG. 1CONT'D

signal peptide. Both N- and C- termini of ORF yet to be elucidated.

ID-100

Clone RS-79

ATGGGACGTAAGTGGGCCAATATTGTTGCCAAAAAGACTGCTAAAGATGG
TGCTAACTCAAAAGTATACGCTAAATTCGGTGTTGAAATATATGTTGCTGC
AAAGCAAGGTGAACCAGACCCCGAGTCAAACTCAGCTCTAAAATTCGTTT
TGGACCGTGCTAAGCAAGCACAAGTTCCAAAGCATGTTATTGATAAAGCG
ATTGATAAAGCCAAAGGAAACACAGATGAAACTTTCGTAGAGGGACGCTA
TGAAGGTTTTGGTCCAAATGGTTCAATGATTATTGTGGATACTTTGACATC
AAATGTTAACCGTACGGCAGCAAATGTACGTACTGCTTACGGTAAGAACG
GTGGCAATATGGGAGCTTCAGGATCGGTATCCTACTTATTTGATAAAAAAG
GTGTCATCGTTTTTGCTGGTGATGATGCTGACACTGTCTTCGAACAATTACT
TGAAGCGGATGTAGACGTAGATGATGTTGAAGCAGAAGAGGGAACAATA
ACAGTTTATACCGCCCCAACAGATCTTCATAAAGGTATCCAAGCACTTCGC
GATAATGGTGTAGAAGAATTCCAAGTTACTGAACTTGAAATGATTCCTCAA
TCAGAAGTAGTATTGGAAGGTGATGACCTTGAAACTTTTGAAAAGCTT

MGRKWANIVAKKTAKDGANSKVYAKFGVEIYVAAKQGEPDPESNSALKFVL
DRAKQAQVPKHVIDKAIDKAKGNTDETFVEGRYEGFGPNGSMIIVDTLTSNV
NRTAANVRTAYGKNGGNMGASGSVSYLFDKKGVIVFAGDDADTVFEQLLEA
DVDVDDVEAEEGTITVYTAPTDLHKGIQALRDNGVEEFQVTELEMIPQSEVVL
EGDDLETFEKL

Sequence description:

A] Length: 654 bp - 218 aa (Partial sequence)
B] Possible Shine Dalgarno sequence upstream of ATG start, no obvious signal peptide

ID-101

Clone RS-80

FIG. 1CONT'D

TTGGAGAAATATTTGAAGAACCCGATTACATGGATTGGATTAGTTCTTGTG
GTTACGTGGTTTTTAACTAAAAGTAGTGAATTTTTGATTTTTGGTGTGTGTG
TCTTGTTGTTAGTATTTGCTAGTCAAAGTGAT

MEKYLKNPITWIGLVLVVTWFLTKSSEFLIFGVCVLLLVFASQSD

Sequence description:

A] Length: 135 bp - 45 aa (partial sequence)
B] Shine Dalgarno sequence upstream of TTG start codon with possible signal peptide evident at N-terminus.

ID-102

Clone RS-81

ATGACACAATCAGATGCATATCTCTCGTTGAACGCGAAGACACGCTTTAGA
GATCGCACAGGTAATTATCATTTTACTTCGGATAAAGAGGCTGTTGAACAA
TATATGATAGAACATGTTGAACCTAATACGATGGTGTTCACATCACTAATT
GAAAAGCTAGATTATTTGGTTTCTAATAACTACTATGAATCGGACCTTCTA
AAACAATATAACCTTGAGTTTATTTGCCAAATTTTTGAGCATGCATACGCT
AAGAAATTTGCTTTTCTAAATTTTATGGGGGCTTTAAAATTTTATAATGCTT
ATGCTCTTAAT

MTQSDAYLSLNAKTRFRDRTGNYHFTSDKEAVEQYMIEHVEPNTMVFTSLIE
KLDYLVSNNYYESDLLKQYNLEFICQIFEHAYAKKFAFLNFMGALKFYNAYA
LN

Sequence description:

A] Length: 318 bp - 106 aa (Partial sequence)
B] Shine Dalgarno sequence present upstream of ATG start codon, no obvious signal peptide

FIG. 1CONT'D

ID-103

Clone 2-11A

ATGGTATTTATGGCAAATAAGAAAAAAACAAAAGGAAAGAAAACCAGAA
GACCTACTAAGGCAGAAATAGAGCGTCAAAGAGCTATTCAAAGGATGATT
ACTGCTCTTGTTTTAACAATTATTCTCTTCTTTGGTATTATCAGATTAGGTA
TTTTTGGTATTACAGTCTATAACGTCATCCGTTTTATGGTAGGTAGCTTGGC
TTACTTATTTATTGCGGCAACTTTAATCTACCTTTATTTCTTTAAATGGTTG
CGAAAGAAAGATAGCTTAGTAGCAGGTTTTTTGATAGCTTCTTTAGGATTA
TTGATTGAGTGGCATGCTTACCTTTTCTCAATGCCTATTTTGAAAGATAAA
GAAATTTTGCGTTCAACTGCTCGATTAATTGTGTCTGATTTAATGCAATTTA
AAATCACTGTTTTTGCCGGTGGAGGTATGTTGGGTGCTTTGATTTACAAGC
CAATTGCTTTTCTCTTTTCTAATATTGGTGCCTATATGATTGGTGTTCTCTTC
ATCATTTTGGGTCTCTTTTTAATGAGTTCTCTGGAAGTTTATGACATCGTCG
AATTTATTAGAGCTTTTAAAAATAAAGTGGCAGAGAAGCACGAGCAAAAT
AAAAAGGAGCGTTTTGCTAAGCGAGAGATGAAAAAAGCAATCGCTGAACA
AGAGCGCATAGAGCGTCAAAAAGCTGAAGAAGAAGCTTATTTAGCTTCGG
TTAATGTAGACCCTGAAACGGGTGAGATTCTAGAGGATCAAGCTGAGGAC
AATTTGGATGATGCGCTACCACCTGAGGTAAGTGAAACATCAACTCCGGT
ATTTGAGCCAGAGATCCTTGCTTATGAGACATCGCCTCAAAATGATCCTTT
ACCAGTAGAGCCGACAATTTATTTAGAAGACTATGATTCGCCGATTCCTAA
TATGAGAGAAAATGATGAGGAAATGGTTTATGATTTAGATGATGATGTAG
ATGATAGTGATATAGAAAATGTCGACTTTACACCTAAAACGACACTGGTTT
ATAAATTACCAACGATAGATTTATTTGCACCAGATAAGCCTAAAAATCAAT
CCAAAGAAAAGGATTTAGTCCGAAAGAATATCAGAGTTTTAGAAGAAACA
TTTAGAAGTTTTGGTATCGATGTAAAAGTAGAACGTGCTGAAATTGGACCA
TCAGTTACTAAATATGAAATTAAACCAGCAGTTGGAGTTCGTGTGAATCGT
ATTTCAAATCTATCTGACGACCTAGCTCTTGCTCTTGCAGCAAAAGATGTG
CGTATAGAAGCACCAATTCCTGGAAAATCATTAATAGGTATTGAAGTTCCT
AACTCAGAAATTGCAACGGTTTCTTTCCGCGAACTTTGGGAACAATCTGAT
GCCAATCCTGAAAACCTTTTAGAAGTACCACTAGGAAAAGCTGTTAACGG
CAATGCTCGCAGTTTTAACTTAGCTAGAATGCCGCATCTTTTGGTAGCTGG
TTCAACTGGTTCAGGTAAATCTGTGGCAGTTAATGGAATTATTTCAAGTAT
TTTGATGAAGGCACGTCCAGATCAAGTTAAGTTTATGATGATTGATCCCAA
AATGGTTGAATTATCTGTTTATAATGATATTCCACATTTATTAATCCCTGTT
GTAACCAATCCGCGTAAAGCAAGTAAGGCACTCCAAAAAGTTGTTGATGA
AATGGAAAATCGATACGAGTTATTTAGCAAAATTGGTGTGCGTAATATAG
CAGGTTATAATACAAAGGTTGAAGAGTTTAATGCTTCCTCTGAGCAAAAAC
AAATGCCTTTGCCTTTAATCGTTGTCATTGTAGATGAATTGGCTGACTTGAT
GATGGTTGCTAGTAAAGAAGTTGAAGATGCTATTATTCGTTTGGGGCAAAA
AGCACGTGCTGCAGGTATCCATATGATTCTTGCAACTCAACGTCCATCCGT

FIG. 1 CONT'D

AGATGTTATTTCTGGTTTGATTAAAGCAAATGTTCCGTCGCGTATTGCATTT
GCTGTTTCAAGTGGTACTGATAGCCGTACGATCCTTGATGAAAATGGTGCT
GAAAAGCTCTTGGGACGGGGTGACATGCTCTTTAAGCCTATTGATGAGAAT
CATCCAGTACGACTACAAGGTTCCTTTATTTCAGATGATGATGTTGAAAGG
ATCGTTGGTTTTATCAAAGACCAAGCCGAGGCTGACTATGATGATGCCTTT
GATCCTGGAGAAGTATCTGAAACAGATAACGGCTCTGGTGGTGGCGGCGG
AGTACCTGAAAGTGATCCTCTTTTTGAAGAAGCCAAGGGACTCGTTTTAGA
GACGCAAAAAGCAAGTGCCTCAATGATTCAACGCCGATTGTCTGTTGGTTT
CAATAGAGCAACAAGACTAATGGAAGAATTAGAAGCAGCGGGGGTTATTG
GTCCAGCAGAAGGAACCAAGCCACGAAAAGTTTTAATGACTCCAACTCCG
AGTGAATAA

MVFMANKKKTKGKKTRRPTKAEIERQRAIQRMITALVLTIILFFGIIRLGIFGIT
VYNVIRFMVGSLAYLFIAATLIYLYFFKWLRKKDSLVAGFLIASLGLLIEWHA
YLFSMPILKDKEILRSTARLIVSDLMQFKITVFAGGGMLGALIYKPIAFLFSNIG
AYMIGVLFIILGLFLMSSLEVYDIVEFIRAFKNKVAEKHEQNKKERFAKREMK
KAIAEQERIERQKAEEEAYLASVNVDPETGEILEDQAEDNLDDALPPEVSETST
PVFEPEILAYETSPQNDPLPVEPTIYLEDYDSPIPNMRENDEEMVYDLDDDVDD
SDIENVDFTPKTTLVYKLPTIDLFAPDKPKNQSKEKDLVRKNIRVLEETFRSFGI
DVKVERAEIGPSVTKYEIKPAVGVRVNRISNLSDDLALALAAKDVRIEAPIPGK
SLIGIEVPNSEIATVSFRELWEQSDANPENLLEVPLGKAVNGNARSFNLARMPH
LLVAGSTGSGKSVAVNGIISSILMKARPDQVKFMMIDPKMVELSVYNDIPHLLI
PVVTNPRKASKALQKVVDEMENRYELFSKIGVRNIAGYNTKVEEFNASSEQK
QMPLPLIVVIVDELADLMMVASKEVEDAIIRLGQKARAAGIHMILATQRPSVD
VISGLIKANVPSRIAFAVSSGTDSRTILDENGAEKLLGRGDMLFKPIDENHPVRL
QGSFISDDDVERIVGFIKDQAEADYDDAFDPGEVSETDNGSGGGGGVPESDPL
FEEAKGLVLETQKASASMIQRRLSVGFNRATRLMEELEAAGVIGPAEGTKPRK
VLMTPTPSE*

Sequence description:

A] Length: 2451 bp - 817 aa (Full-length gene)
B] Shine Dalgarno sequence present upstream of ATG start codon, possesses a potential signal peptide

ID-104

Clone 2-18/22b

FIG. 1 CONT'D

ATGTCACAAGAGCAAGGAAAAATTTATATTGTAGAAGATGATATGACGAT
TGTGTCACTTTTAAAAGATCATTTATCAGCTAGCTATCATGTCTCTAGTGTC
AGCAATTTTCGTGATGTGAAACAAGAAATTATCGCATTTCAACCCGATTTG
ATACTAATGGATATTACGTTACCCTATTTTAATGGTTTTTACTGGACTGCAG
AATTGCGTAAGTTTTTAACAATTCCTATTATTTTCATTTCATCTAGTAATGA
TGAAATGGATATGGTTATGGCATTAAATATGGGGGGTGATGACTTTATTTC
AAAACCATTCTCTCTAGCTGTATTAGATGCTAAGCTAACTGCTATTTTAAG
GAGAAGTCAACAATTTATCCAACAGGAATTAACTTTTGGGGGATTTACGTT
GACAAGAGAAGGGTTATTGTCTAGCCAAGATAAAGAGGTTATTTTATCGC
CAACAGAAAATAAAATCCTATCTATCTTGCTCATGCATCCTAAACAAGTAG
TCTCAAAAGAGTCTCTATTAGAGAAACTTTGGGAAAATGATAGTTTTATTG
ATCAAAATACACTTAATGTTAATATGACACGCTTACGTAAAAAAATTGTCC
CAATAGGTTTTGATTACATTCATACAGTGAGAGGAGTTGGGTATTTACTAC
AATGA

MSQEQGKIYIVEDDMTIVSLLKDHLSASYHVSSVSNFRDVKQEIIAFQPDLILM
DITLPYFNGFYWTAELRKFLTIPIIFISSSNDEMDMVMALNMGGDDFISKPFSLA
VLDAKLTAILRRSQQFIQQELTFGGFTLTREGLLSSQDKEVILSPTENKILSILLM
HPKQVVSKESLLEKLWENDSFIDQNTLNVNMTRLRKKIVPIGFDYIHTVRGVG
YLLQ*

Sequence description:

A] Length: 669 bp - 223 aa (full-length gene sequence)
B] Shine Dalgarno sequence present upstream of a GTG start codon. Was not identified directly by LEEP. This gene was found upstream of gene ID-10 described in WO 00/06736.

ID-105

Clone 2-20

ATGTATCAAACTCAGACAAATAAGGAAAAATTTGTTTTATTTTTGAAATTA
TTTATCCCAGTATTGATTTATCAATTTGCTAATTTTTCAGCTACTTTTATTGA
TTCGGTTATGACTGGACAGTATAGTCAGCTACATTTGGCAGGTGTGTCAAC
TGCTAGTAATTTATGGACTCCGTTTTTCGCTTTATTAGTAGGTATGATTTCA
GCATTAGTACCAGTAGTTGGTCAACATTTGGGTAGAGGAAATAAAGAACA
AATTCGCACAGAATTTCATCAATTTCTATATTTAGGTTTGATACTGTCCTTA
ATATTATTTTTAATCATGCAATTTATTGCTCAACCTGTCTTGGGGAGTTTGG

FIG. 1CONT'D

GTTTAGAAGATGAAGTTCTAGCAGTTGGTCGTGGTTATTTAAATTATATGT
TGATTGGAATCATGCCGCTGGTGTTGTTTAGCATTTGCCGTTCATTCTTTGA
TGCATTGGGGTTAACAAGGTTATCTATGTATCTGATGCTTTTAATTCTACCC
TTTAATTCATTTTTTAATTATATGCTTATCTACGGTAAATTTGGTATGCCTA
GACTAGGAGGTGCGGGGGCAGGTCTTGGAACTTCTTTAACTTATTGGGCTA
TTTTTATTGGTATTATTATTGTGATGTCACTTCATCCTCAAATTAAAACATA
TCATATATGGACTCTGGAAAGAATAAAAGCTCCTTTGATTATTGAAGATAT
TCGATTGGGATTACCGATTGGTTTACAAATTTTTGCAGAAGTTGCAATTTTT
GCAGTAGTAGGCTTATTCATGGCAAAATTTTCTTCAATCATTATTGCAGCA
CATCAGGCTGCTATGAATTTTTCATCATTAATGTATGCATTTCCTTTAAGTA
TTTCCACTGCTCTAGCTATTACAATATCGTTTGAAGTAGGGGCAGAGCGCT
TTCAGGACGCAACCACTTATAGTAGGATAGGACGCTTAACAGCGGTAGGG
ATTACATCAGGAACCTTACTATTTTTATTTCTATTTCGTGAGAATGTAGCAG
CAATGTATAATAGTGCCCCTCACTTTGTCGCTATTACAGCTCAATTCCTAAC
TTATAGTCTCTTTTTCCAGTTTGCAGATGCTTATGCAGCTCCTGTACAGGGG
ATTTTACGAGGCTATAAGGATACAACAAAACCATTTATGATCGGTGCGGG
CTCTTATTGGTTATGTGCTTTGCCATTAGCGGTTATCTTAGAAAAAAATAG
CCAGTTAGGTCCGTTTGCCTATTGGATTGGTTTAATCACAGGTATTTTTGTT
TGTGGTCTATTTCTAAACCAACGTCTGCAAAAGATTAAGAAGTTGTATTAT
TAA

MYQTQTNKEKFVLFLKLFIPVLIYQFANFSATFIDSVMTGQYSQLHLAGVSTAS
NLWTPFFALLVGMISALVPVVGQHLGRGNKEQIRTEFHQFLYLGLILSLILFLI
MQFIAQPVLGSLGLEDEVLAVGRGYLNYMLIGIMPLVLFSICRSFFDALGLTRL
SMYLMLLILPFNSFFNYMLIYGKFGMPRLGGAGAGLGTSLTYWAIFIGIIIVMS
LHPQIKTYHIWTLERIKAPLIIEDIRLGLPIGLQIFAEVAIFAVVGLFMAKFSSIIIA
AHQAAMNFSSLMYAFPLSISTALAITISFEVGAERFQDATTYSRIGRLTAVGITS
GTLLFLFLFRENVAAMYNSAPHFVAITAQFLTYSLFFQFADAYAAPVQGILRG
YKDTTKPFMIGAGSYWLCALPLAVILEKNSQLGPFAYWIGLITGIFVCGLFLNQ
RLQKIKKLYY*

Sequence description:

A] Length: 1341 bp - 447 aa (full length gene)
B] Shine-Dalgarno sequence present upstream of ATG start codon, There is a potential signal peptide sequence

ID-106

FIG. 1 CONT'D

Clone 2-4A

TTGCTAGTTTCTTCTCTAGTTTCTTGTTCATTTTTTCTTGTCATTTCGTCGTT
GTCTTCATCAACACGAAATAAGTCTATAAACTTATCAAATAATTTCATAGA
CTTATTATATCAATTTTCAATAAAATGCTATAATAAAACCATGTCATTTTCA
TTAAAAATTAGAAATCCATACGGTGAACATACCGTTAAAGAACTCCTTGA
AGATTATTTTTTGATTCCACGTAAGATTAGACATTTTTTGCGTGTTAAAAAA
CATGTACTTATAAACAATGAATTCATTAATTGGCAAACTGTCGTCCAAGAA
AACGATACTATTACCTTAATCTTTGATGATGAGGATTACCCTACTAAAAAA
ATTCCTCTGGGCAGAGCAGAGCTTATTGATTGTCTTTATGAGGATGAACAT
CTTATTATCGTTAATAAACCTGAAGGTATGAAAACTCACGGTAACCAACCA
AATGAAATAGCACTGTTAAATCATGTATCTGCCTATTCTGGACAAACATGC
TATGTTGTTCATCGCCTAGATATGGAGACCAGTGGAGCTGTTTTATTTGCT
AAAAATCCATTTATACTTCCCCTTATCAATCAACGCTTAGAACGAAAAGAA
ATTTGGCGTGAATATTGGGCTTTAGTTGAAGGAAAATTTTCACCTAAGCAT
CAAGTTTTGAGAGACAAAATTGGACGGAACCGTCATGACAGACGTAAACG
AATCATTGATTCTAAAAACGGTCAACATGCTATGACAATCATTGACGTTTT
GAAGTATATCCAAAATAGTAGTCTCATAAAATGCCGACTGGAAACCGGAA
GAACCCATCAAATTCGCATTCACTTATCTCATCACGGACATCCTTTAATAG
GAGATCCCCTCTACAACCCTTCTTCTAATAATGAAAGGTTAATGCTACACG
CTCACCGATTGACTCTATCCCATCCATTAACTTGCGAAACTATTAGCGTAG
AGGCCCCTTCATCTACTTTCGAGAAGGTTTTAAACAATTATAAAAAAGGAG
TTGGATAA

MLVSSLVSCSFFLVISSLSSSTRNKSINLSNNFIDLLYQFSIKCYNKTMSFSLKIR
NPYGEHTVKELLEDYFLIPRKIRHFLRVKKHVLINNEFINWQTVVQENDTITLIF
DDEDYPTKKIPLGRAELIDCLYEDEHLIIVNKPEGMKTHGNQPNEIALLNHVSA
YSGQTCYVVHRLDMETSGAVLFAKNPFILPLINQRLERKEIWREYWALVEGKF
SPKHQVLRDKIGRNRHDRRKRIIDSKNGQHAMTIIDVLKYIQNSSLIKCRLETG
RTHQIRIHLSHHGHPLIGDPLYNPSSNNERLMLHAHRLTLSHPLTCETISVEAPS
STFEKVLNNYKKGVG*

Sequence description:

A] Length: 1029 bp - 343 aa (Full length gene sequence)
B] No obvious Shine-Dalgarno sequence upstream of the putative TTG start codon. Possesses a potential leader peptide sequence.

FIG. 1 CONT'D

ID-107

Clone 2-54

GAACTAAATGCAACTCAACCTAATAATAGAACTACCTATATTATACCCGAA
AGCAGTCATTCCATTGCAGAACAACAGAGATTCCTGATAGAATCAAAGGG
TTCTTCGGTTGCATTACTTAATAGCGATGAATTTAGAAAGACAGCGGGAGA
GGATAGAGGTTTTGAAAGGGATAAGTTGAGGTCTTTGGATATCATTCCTAA
GGGAGATTTATCGACAAGTAATGTCATAGGTAATACGGACATTGCTAGTC
AGATATCGTTGGGCTTTAAAAAGAATGCGATGCAGGAACACCATCTTACT
AAAACATTCTCTCAAAAGGATGGAAAGTTATCGTCTGTTATAGAGGGGAT
GCTTGCTATTGGCAAAGAGAAAGTAGAGAAAGAAATAAAATATAGTGGTA
ATTTATGGCAAAAATTAAAAGCTAAGGCACACTGCCTTGTTTGCTGTGTTG
ATAATTTGAATTTTGAAGATATAAAATCTTATTTTCAATATTATTGTCATCT
AAACCATCAGCTCAAATTACCTAAAGGTGCTATACTTTCTGCTAAAACAGA
AGTATATAGGGGAGGAGATTTTGGGAGAAAAAATAAAGATAATGTGTTTG
GTTACCGTATCCCCTCATTATTGAAAACCCAAAAAGGAACTTTACTTGCGG
GAGCTGATGAAAGAATTGAGCAAGCTTGTGATTGGGGAAACATAGGAATG
GTTATTCGCCGTAGTGAGGATGATGGTGTCACTTGGGGAAAAAGAGAAAC
TATTGTCAATCTCCGTAATAACCCTAGAGTTCCGCTAGTTACTAGTGGTGA
CTATAGTGGCTCACCTATTAATATGGATATGGCATTAGTTCAAGATACTAG
CTCCAAGACGAAACGTATTTTTTCAATATATGATATGTTTCCAGAAGGAAG
AGGCGTTATTAGTATTGCTAACACACCTGAAAAAGAATATACCCAAATCG
GAGGACAGTCTTATCTTAATTTATATAATAATGGAAAGAAATCGAAGGTTT
TTACTATCCGTGACAAAGGTATTGTATATAATTTTAAAGGGAAAAAGACTG
ATTATCATGTTATAACAGAAACTACTAAAAGTGACCATTCAAATCTAGGGG
ATATTTATAAGGGAAAACAGCTACTTGGAAATATATATTTTACAAAACATA
AAACGTCACCATTTCGTTTAGCAAAATCAAGCTATGTGTGGATGTCATATA
GCGATGATGATGGTAGGACATGGTCATCACCTAGAGATATAACAGCAAGT
CTTCGTCAGAAAGGCATGAAATTTTTGGGAATAGGACCTGGAAAAGGTAT
AGTTTTAAAATGGGGGCCACACGCTGGTCGTATTATTATTCCTGCCTATTCT
ACGAATTGGAAATCTCATCTAAGAGGTTCACAATCTTCACGCCTAATTTAT
TCAGACGACCATGGAAAAACGTGGCATACTGGAAAAGCAGTTAATGATAA
CCGTATACTTTCTAATGGTGAAAAAATTCACTCCTTAACAATGGATAATAA
AAAAGAACAAAATACAGAATCCGTACCCGTTCAATTGAAAAATGGGGACA
TTAAGTTATTTATGAGGAATCTAACTGGTAACCTAGAAGTAGCCACAAGTA
AAGACGGCGGGGAGACTTGGCAAAACCATGTTAAACGATATAAGGAAATT
CATGATGCTTACGTCCAACTATCAGCTATTCGCTTTGAGCATGACAAAAAA
GAGTATATTTTATTAGTGAATGCTAATGGGCCAGGGAAGAAGTGCCAAGA
TGGATATGCACGTCTAGCGCAAGTTAATCGAAATGGTAGTTTTAAGTGGTT
ATATCACCATCACATTCAAGATGGTTCGTTTGCTTACAACTCTGTTCAACA
ACTTAATAATGATCAATTTGGTGTCCTTTATGAACATAGAGAAAAACATCA

FIG. 1 CONT'D

AAATAGTTTTACTTTAAATTACAAAGTTTTTAATTGGAGTTTTCTTAGTCAA
AATACAGAGAAGCAAGGCACTTTATGGGAGAAAATGGCAGCAAATTGGCA
TGTTTTGTTTAAATTTTATTTATGA

ELNATQPNNRTTYIIPESSHSIAEQQRFLIESKGSSVALLNSDEFRKTAGEDRGF
ERDKLRSLDIIPKGDLSTSNVIGNTDIASQISLGFKKNAMQEHHLTKTFSQKDG
KLSSVIEGMLAIGKEKVEKEIKYSGNLWQKLKAKAHCLVCCVDNLNFEDIKS
YFQYYCHLNHQLKLPKGAILSAKTEVYRGGDFGRKNKDNVFGYRIPSLLKTQ
KGTLLAGADERIEQACDWGNIGMVIRRSEDDGVTWGKRETIVNLRNNPRVPL
VTSGDYSGSPINMDMALVQDTSSKTKRIFSIYDMFPEGRGVISIANTPEKEYTQI
GGQSYLNLYNNGKKSKVFTIRDKGIVYNFKGKKTDYHVITETTKSDHSNLGDI
YKGKQLLGNIYFTKHKTSPFRLAKSSYVWMSYSDDDGRTWSSPRDITASLRQ
KGMKFLGIGPGKGIVLKWGPHAGRIIIPAYSTNWKSHLRGSQSSRLIYSDDHG
KTWHTGKAVNDNRILSNGEKIHSLTMDNKKEQNTESVPVQLKNGDIKLFMRN
LTGNLEVATSKDGGETWQNHVKRYKEIHDAYVQLSAIRFEHDKKEYILLVNA
NGPGKKCQDGYARLAQVNRNGSFKWLYHHHIQDGSFAYNSVQQLNNDQFG
VLYEHREKHQNSFTLNYKVFNWSFLSQNTEKQGTLWEKMAANWHVLFKFYL
*

Sequence description:

A] Length: 2052 bp - 684 aa (partial gene sequence)
B] N-terminus has yet to be determined

ID-108

Clone 2-61

ATGCCTAAATTAATCGTATCTTTCCTCTGCATTTTATTATCCCTGACTTGTG
TAAACTCTGTGCAAGCTGAAGAACATAAAGATATTATGCAAATTACCCGA
GAAGCCGGATATGATGTTAAAGATATTAATAAACCTAAAGCGTCTATCGTT
ATTGACAATAAAGGTCATATTTTGTGGGAAGATAACGCCGATTTAGAACGT
GATCCCGCTAGCATGTCTAAAATGTTTACTTTATATTTACTATTTGAAGACT
TAGCTAAAGGAAAAACAAACCTCAACACCACAGTGACTGCAACAGAAACA
GACCAAGCCATAAGTAAGATTTATGAAATTAGTAATAACAATATTCATGCT
GGGGTTGCTTATCCTATTCGTGAACTGATTACTATGACGGCTGTCCCGTCA
TCTAATGTAGCAACTATTATGATTGCTAACCACTTATCACAAAACAATCCT
GACGCCTTTATTAAACGAATCAATGAAACCGCCAAGAAACTCGGTATGAC
AAAAACTCACTTTTATAACCCCAGTGGGGCGGTAGCGAGTGCTTTTAATGG
ACTTTACTCCCCAAAAGAATACGATAACAATGCTACTAACGTTACGACTGC

FIG. 1CONT'D

ACGTGATCTATCAATTTTAACCTATCATTTCCTTAAAAAATACCCTGATATA
CTGAACTATACAAAATATCCTGAAGTCAAGGCCATGGTCGGAACTCCTTAT
GAAGAAACATTTACAACTTATAACTACTCTACCCCCGGCGCTAAATTTGGA
TTAGAAGGAGTAGATGGCTTAAAAACTGGTTCTAGCCCTAGCGCTGCTTTT
AATGCCTTAGTTACAGCTAAACGCCAGAATACTCGCTTGATAACTGTGGTT
TTAGGAGTTGGCGATTGGTCAGACCAAGACGGAGAGTACTATCGTCATCC
GTTTGTCAACGCTCTTGTAGAAAAAGGTTTTAAAGACGCTAAAAATATTTC
TTCTAAAACTCCTGTATTAAAAGCCGTTAAACCTAAAAAAAGAAGTTACTAA
AACCAAAACTAAATCTATTCAAGAACAGCCTCAAACAAAAGAACAGTGGT
GGACAAAAACAGATCAATTTATCCAATCACATTTTGTATCTATTTTAATTG
TTCTGGGCACCATCGCTAGCCTTTGTCTTTTAGCTGGGATAGTATTACTTAT
AAAGCGCTCTAGATAA

MPKLIVSFLCILLSLTCVNSVQAEEHKDIMQITREAGYDVKDINKPKASIVIDN
KGHILWEDNADLERDPASMSKMFTLYLLFEDLAKGKTNLNTTVTATETDQAI
SKIYEISNNNIHAGVAYPIRELITMTAVPSSNVATIMIANHLSQNNPDAFIKRINE
TAKKLGMTKTHFYNPSGAVASAFNGLYSPKEYDNNATNVTTARDLSILTYHF
LKKYPDILNYTKYPEVKAMVGTPYEETFTTYNYSTPGAKFGLEGVDGLKTGS
SPSAAFNALVTAKRQNTRLITVVLGVGDWSDQDGEYYRHPFVNALVEKGFK
DAKNISSKTPVLKAVKPKKEVTKTKTKSIQEQPQTKEQWWTKTDQFIQSHFVS
ILIVLGTIASLCLLAGIVLLIKRSR*

Sequence description:

A] Length: 1188 bp - 396 aa (full length gene)
B] Shine Dalgarno sequence present upstream of ATG start codon, possesses a potential signal peptide

ID-109

Clone 45

ATGACTGAAAAATATTATAATTGGGCAACGCTTGGAACCGGCGTTATTGCC
AACGAATTAGCCCAAGCACTGGAAGCACGTGGACAAAAATTATATTCTGT
AGCTAATAGAACTTACGACAAAGGACTTGAATTTGCTAACAAATATGGTA
TCCAAAAAGTTTATGATCACATAGATCAAGTATTTGAAGACCCTGAAGTGG
ATATCATTTATATCTCTACTCCCCACAATACTCACATCTCATTTTTACGAAA

FIG. 1CONT'D

GGCTTTAGCAAATGGTAAGCACGTTCTTTGCGAAAAATCTATTACTTTAAA
TAGTACTGAGCTTAAAGAAGCCATAGATTTAGCCGAAACTAACCATGTTGT
CTTAGCTGAAGCCATGACTATTTTTCATATGCCAATTTACCGCCAATTAAA
AACATTAGTTGATAGTGGAAAATTAGGACCGTTAAAAATGATTCAAATGA
ATTTCGGAAGTTATAAAGAATATGATATGACTAACCGTTTTTTCAGTCGTG
ACCTAGCAGGCGGTGCTTTGCTGGACATTGGTGTTTATGCACTTTCTTGTAT
TCGCTGGTTTATGTCAGAAGCACCTCACAACATTACCTCTCAAGTTACATT
TGCACCAACAGGGGTTGATGAACAAGTTGGTATCCTACTAACCAACCCAG
CAAATGAGATGGCGACTGTCAGCCTTAGTTTACATGCAAAACAACCTAAA
CGAGCAACTATCGCTTACGATAAAGGCTACATTGAACTTTTTGAATATCCG
CGAGGACAAAAGGCAGTTATTACTTATACTGAGGATGGGCATCAAGATAT
TATCGAAGCTGGCAAAACTGAAAATGCTCTCCAATATGAGGTAGCTGATA
TGGAAGAAGCCATTTCAGGAAAAACTAACCACATGTACTTAAACTATACC
AAAGATGTTATGGATATCATGACACAGCTACGTCAAGAATGGGGATTTAC
CTACCCAGAAGAAGAAAAATGA

MTEKYYNWATLGTGVIANELAQALEARGQKLYSVANRTYDKGLEFANKYGI
QKVYDHIDQVFEDPEVDIIYISTPHNTHISFLRKALANGKHVLCEKSITLNSTEL
KEAIDLAETNHVVLAEAMTIFHMPIYRQLKTLVDSGKLGPLKMIQMNFGSYK
EYDMTNRFFSRDLAGGALLDIGVYALSCIRWFMSEAPHNITSQVTFAPTGVDE
QVGILLTNPANEMATVSLSLHAKQPKRATIAYDKGYIELFEYPRGQKAVITYT
EDGHQDIIEAGKTENALQYEVADMEEAISGKTNHMYLNYTKDVMDIMTQLR
QEWGFTYPEEEK*

Sequence description:

A] Length: 984 bp - 328 aa (full length gene)
B] Shine Dalgarno sequence present upstream of ATG start codon, possesses a potential signal peptide

ID-110

Clone 2-2

GTGTATTCTCCTGTTAAATCTTCTAAAGGAAAAGTGATATTGTTAAAAAGT
GATTTTCTAAAGAGCTTCATAGAAAGGAGAGGAAATATTTGTTTT

MYSPVKSSKGKVILLKSDFLKSFIERRGNICF

FIG. 1CONT'D

Sequence description:

A] Length: 96 bp - 32 aa (partial sequence)
B] GTG start codon - no obvious Shine-Dalgarno sequence
Possesses a potential signal peptide

ID-111

Clone 2-3

AAATACTGTATCATTGCAACCTCAAATGCAGGTTTTGGAAACGAAGCATTT
ACAGGTGACAGCGATAAAGACTTGAAAATTATGGAACGAATTTCTCCATA
TTTCCGTCCAGAATTTCTAAATCGTTTCAATGGTGTTATTGAATTCTCTCAC
CTAAGCAAAGATGACTTAAGCGAAATTGTAGATTTGATGCTTGATGAAGTT
AACCAAACAATTGGCAAAAAAGGAATTGACCTTGTGGTAGATGAAAATGT
TAAATCACACTTAATTGAACTGGGTTATGACGAAGCAATGGGAGTACGTC
CATTGCGCCGTGTCATCGAGCAAGAAATTCGAGATCGCATCACAGACTACT
ATCTCGATCATACAGACGTTAAACACCTAAAAGCTAATTTGCAAGATGGCC
AAATCGTCATTTCTGAAAGATAA

KYCIIATSNAGFGNEAFTGDSDKDLKIMERISPYFRPEFLNRFNGVIEFSHLSKD
DLSEIVDLMLDEVNQTIGKKGIDLVVDENVKSHLIELGYDEAMGVRPLRRVIE
QEIRDRITDYYLDHTDVKHLKANLQDGQIVISER*

Sequence description:

A] Length: 429 bp - 143 aa (partial sequence)
B] N-terminus yet to be elucidated. This gene was not in frame with nuc

ID-112

Clone 2-5

FIG. 1 CONT'D

ATGTCAATGAATTTTTCATTTTTACCACAATATTGGTCCTATTTTAATTATG
GTGTGATGGTAACCATTATGATTTCAACATGTGTTGTTTTTTTTGGAACTAT
TATAGGCGTGTTAATTGCTTTAGTAAAGCGTACTAATTTACATTTTCTCACA
ATATTAGCTAATTTCTATGTATGGGTATTTCGTGGGACACCGATGGTAGTT
CAAATTATGATTGCTTTCGCATGGATGCATTTTAACAATTTACCAACAATT
AGCTTTGGTGTTTTAGATTTAGATTTTACACGACTTTTACCTGGTATCATTA
TCATTTCCTTAAATAGTGGTGCCTATATTTCGGAAATTGTACGTGCAGGGA
TTGAGGCTGTACCATCTGGACAAATAGAAGCAGCTTACTCGTTGGGGATTC
GACCTAAAAATACACTTCGCTATGTTATCTTACCCCAAGCTTTTAAAAATA
TTTTACCTGCTCTAGGGAATGAATTTATTACAATTATTAAAGATAGTGCTCT
CCTTCAAACTATTGGTGTCATGGAATTATGGAACGGAGCACAATCAGTTGT
AACGGCTACTTACTCACCAGTTGCACCGTTATTATTTGCAGCATTTTACTAT
TTAATGTTGACAACGATTCTCTCAGCTTTGTTAAAACAAATGGAGAAATAT
CTTGGGAAAGGGGTAAAAATAGATGGTTGA

MSMNFSFLPQYWSYFNYGVMVTIMISTCVVFFGTIIGVLIALVKRTNLHFLTIL
ANFYVWVFRGTPMVVQIMIAFAWMHFNNLPTISFGVLDLDFTRLLPGIIIISLNS
GAYISEIVRAGIEAVPSGQIEAAYSLGIRPKNTLRYVILPQAFKNILPALGNEFITI
IKDSALLQTIGVMELWNGAQSVVTATYSPVAPLLFAAFYYLMLTTILSALLKQ
MEKYLGKGVKIDG*

Sequence description:

A] Length: 699 bp - 233 aa (full length gene)
B] Shine-Dalgarno sequence preceded the 'ATG' start codon. Possesses a potential leader peptide sequence.

ID-113

Clone 2-7

ATGAAAGACCTATTACGAAATAGTCTAGAGCAAAGTGGAAATTTAAGTTT
TCAAGATATGATTTTACATATTCTTGTAGCAGCTTTATTGAGTGTAGTTATT
TATGTTTCCTATGCTTATACGCATAGTGGAACTGCCTATAGTAAAAAGTTT
AATGTTTCATTAATGACATTGACGGTCTTGACTGCAACAGTAATGACCGTT
ATTGGTAATAATGTAGCCTTGTCATTGGGTATGGTCGGTGCCTTGTCAGTT
GTTCGTTTTAGGACAGCCATAAAAGATTCAAGAGATACAGTTTATATTTTT
TGGACCATAGTTGTTGGTATCTGTTGTGGTGTCGGTGACTATGTGGTAGCT

FIG. 1CONT'D

GCATTAGGAAGTAGCGTTATCTTTATCTTATTATGGGTTATGGGACGTGTT
AAAAACGAGAATCGTATGTTATTGATTGTGAAGTGCGATAGAACACTAGA
AGTTGATTTAGAAGGAATTTTCTTCCAATATTTTGACGGAAAAGCTGTTCA
GCGTGTTAAAAATTCAACAACTAATACTATTGAAATGATTTTCGAAATCTC
TAGAAAAGATTACGATAAGCAACTCCATGTAGATAATCAGTTAACTGAAA
AAGTGTACCAATTGGGAAATATTGATTATTTCAACATTGTTAGCCAAAGCG
ACGAAATCAATGGGTAG

MKDLLRNSLEQSGNLSFQDMILHILVAALLSVVIYVSYAYTHSGTAYSKKFNV
SLMTLTVLTATVMTVIGNNVALSLGMVGALSVVRFRTAIKDSRDTVYIFWTIV
VGICCGVGDYVVAALGSSVIFILLWVMGRVKNENRMLLIVKCDRTLEVDLEGI
FFQYFDGKAVQRVKNSTTNTIEMIFEISRKDYDKQLHVDNQLTEKVYQLGNID
YFNIVSQSDEING*

Sequence description:

A] Length: 678 bp - 226 aa (full-length gene)
B] ATG start codon is preceded by a Shine-Dalgarno sequence-Possesses a potential leader peptide sequence

ID-114

Clone 2-8

AAAAATTCATTTTAGATTCATTTTACGACTATATACTCAGAAGTACCAAAC
CTAATCCAAGGTTTGAAAAAAGAAAGAAGGAAGTCAGTATGACAAACTAT
AAAAACAACTTTAAAGATGAGGCTATACGTGTTGAAGAGACAACAAAAGA
ATCATTTTACGATGTTGATATTGCCTTGTTTTCAGCTGGTGGATCTATTTCA
GCAAAGTTCGCTCCTTATGCAGTAAAGTCTGGAGCAGTTGTAGTAGATAAC
ACGTCATATTTTCGTCAGAATCCTGATGTTCCACTAGTTGTTCCTGAAGTAA
ATGCTCATGCCATGATTGGTCATAATGGTATCATAGCTTGTCCCAATTGTTC
TACTATTCAAATGATGATTGCTTTAGAGCCCATTCGTCAAAAATGGGGGAT
AGAGCGTGTTATAGTTTCCACCTATCAAGCTGTTTCGGGTTCAGGTGCACG
TGCTGTTGAAGAAACTAAGGAACAGTTGAGACAAGTTTT

KFILDSFYDYILRSTKPNPRFEKRKKEVSMTNYKNNFKDEAIRVEETTKESFYD
VDIALFSAGGSISAKFAPYAVKSGAVVVDNTSYFRQNPDVPLVVPEVNAHAMI
GHNGIIACPNCSTIQMMIALEPIRQKWGIERVIVSTYQAVSGSGARAVEETKEQ
LRQV

FIG. 1 CONT'D

Sequence description:

A] Length: 499 bp - 165 aa (partial sequence)
B] N-terminus has yet to be determined

ID-115

Clone 2-9

ATGACAAATGAATTGATAATGCAAGCTTTTGAGTGGTATTTACCTAGTGAT
GGGAATCACTGGAAGAAATTAGAGGAGTCTATATCAGACCTTAAAAAACT
TGGAATTAGTAAAATCTGGTTACCACCAGCATTTAAGGGAACTAGCAGTG
ATGATGTAGGATATGGTGTTTATGATCTCTTTGATTTAGGAGAATTTGACC
AGAATGGAACAATTAGAACAAAATATGGTAGGAAAGAAGAGTATCTAAA
GCTTATTAAGTCGTTAAAGGCAAATGGCATTAAACCGTTTGCAGATATCGT
TCTTAACCATAAAGCCAATGGTGATCATAAAGAAAAATTTCAAGTCATCA
AAGTCAATCCTGAAAATCGTCAAGAAGCATTAAGTGAACCCTATGAGATT
GAAGGATGGACGGGATTTGATTTCCCAGGTAGACAGGGTGAGTACAATGA
TTTT

MTNELIMQAFEWYLPSDGNHWKKLEESISDLKKLGISKIWLPPAFKGTSSDDV
GYGVYDLFDLGEFDQNGTIRTKYGRKEEYLKLIKSLKANGIKPFADIVLNHKA
NGDHKEKFQVIKVNPENRQEALSEPYEIEGWTGFDFPGRQGEYNDF

Sequence description:

A] Length: 456 bp - 152 aa (partial sequence)
B] ATG start codon is preceded by a Shine-Dalgarno sequence, no leader peptide sequence.

ID-116

Clone 2-10

FIG. 1 CONT'D

ATGGAGGTTCTTATGAAGAAAGTGTTAGTAAGTAGTCTTTTGGTTTTAGGG
ATTACGATAACGTTACAACCAGTAGTTGAGGCTAAGGGGCCAAAAGTAGC
TTATACACAAGAGGGAATGACTGCTCTTTCGGACACAAATAAAGATAAAG
TCACTACTATTTCTATTGACGAGATTCAAAAAAGCTTAGAAGGTAAGAAGC
CGATTACTGTTAGTTTTGATATTGATGATACACTGCTTTTCAGTAGTCAATA
TTTTCAATATGGTAAAGAATATGTAACTCCTGGATCGTTTGATTTTCTTCAT
AAACAAAAATTCTGGGATCTTGTTGCAAAACGAGGAGATCAAGATTCCAT
TCCCAAAGAATATGCTAAAAAATTAATTGCTATGCATCAAAAACGAGGAG
ATAAAATTGTTTTTATAACAGGTAGGACAAGAGGGTCAATGTATAAGGAG
GGCGAGGTTGATAAAACAGCTAAAGCCTTAGCTAAAGATTTTAAATTTGTA
CCATCTGAT

MEVLMKKVLVSSLLVLGITITLQPVVEAKGPKVAYTQEGMTALSDTNKDKVT
TISIDEIQKSLEGKKPITVSFDIDDTLLFSSQYFQYGKEYVTPGSFDFLHKQKFW
DLVAKRGDQDSIPKEYAKKLIAMHQKRGDKIVFITGRTRGSMYKEGEVDKTA
KALAKDFKFVPSD

Sequence description:

A] Length: 516 bp - 172 aa (partial sequence)
B] ATG start codon is preceded by a Shine-Dalgarno sequence, Possesses a leader peptide sequence.

ID-117

Clone 2-17

ATGCTTAAAAGATTATTTACTGAAGATGGGGAATTGACAAAGATTAGTCGT
CGTTTCGTTTGGATGTTAGTGGTTATCTATTGTCTTATTATTGTCAGGATGT
GTTTTGGGCCTCAAATTATGATTGAGGGGGTATCAACTCCGAATGTTCAGC
GCTTCGGAAGAATTGTAGCTCTTTTAGTACCATTTAATTCTTTTCGTAGTTT
AGATCAGCTAACTAGCTTTAAAGAGATTCTTTGGGTTATTGGTCAAAATGT
AGTGAATATTTTACTGCTGTTTCCTCTCATTATAGGGTTACTATCCCTAAAG
CCAAGTTTACGGAAATATAAAAGCGTTATATTACTTGCTTTCTTGATGTCTC
TTTTCATAGAGTGTACTCAAGTTGTTTTAGATATTTTAATAGATGCTAATCG
GGTTTTTGAAATCGACGATCTATGGACAAATACCTTAGGCGGTCCTTTCGC
CCTATGGAGTTATCGAAACATAAAAGGTTGGCTTCTAACTATTAGAAAATG
A

FIG. 1 CONT'D

MLKRLFTEDGELTKISRRFVWMLVVIYCLIIVRMCFGPQIMIEGVSTPNVQRFG
RIVALLVPFNSFRSLDQLTSFKEILWVIGQNVVNILLLFPLIIGLLSLKPSLRKYK
SVILLAFLMSLFIECTQVVLDILIDANRVFEIDDLWTNTLGGPFALWSYRNIKG
WLLTIRK*

Sequence description:

A] Length: 516 bp - 172 aa (full-length gene)
B] ATG start codon is preceded by an Shine-Dalgarno sequence. Possesses a potential leader peptide sequence. C-terminus need further confirmation.

ID-118

Clone 3-3

ATGAAAAAGCTTACTTTTATTTGGGATTTAGATGGGACATTAATAGATTCG
TATGTACCAATTATGGAAGCTCTTGAAGAAACCTATCGTCATTTTGGCTTA
ATATTTGATAAAGAATTAATCCATGAATATATTTTACAGGAATCAGTGGGG
CAATTATTGGTAAACCTTTCAGAGGAAGAGCAAATACCTCATGAAAAACT
GAAAGCATATTTTACAAAAGAACAAGAAAGTCGAGATTCTAAAATACATT
TAATGCCATATGCAAAAGAGATTTTAGAATGGACCAAAGAACAAGATATT
CCCAATTTTATGTATACACATAAAGGAGCAAGTACGCATTCAGTGTTGGAA
ACCTTGCAGATCTCTCATTATTTTGATGAAATTTTAACTGGTGTTTCGGGAT
TCGAGCGAAAACCACATCCACAAGGGATTAATTATTTAGTTAAACGATATT
CTTTAGATAAATCAATGACTTATTACATAGGAGATCGTCCACTAGATTTGG
AGGTTGCTCAAAATGCTGGTATAAAATCCATAAACTTAAGGTTAGAGAATT
CCAAAGAAAACTATAATATTTCAAGTCTCAAAGATATAATATCACTTGATT
TCACTCGTTTGGATTAA

MKKLTFIWDLDGTLIDSYVPIMEALEETYRHFGLIFDKELIHEYILQESVGQLL
VNLSEEEQIPHEKLKAYFTKEQESRDSKIHLMPYAKEILEWTKEQDIPNFMYTH
KGASTHSVLETLQISHYFDEILTGVSGFERKPHPQGINYLVKRYSLDKSMTYYI
GDRPLDLEVAQNAGIKSINLRLENSKENYNISSLKDIISLDFTRLD*

Sequence description:

A] Length: 627 bp - 209 aa (Possible Full-length gene)

FIG. 1CONT'D

B] ATG start codon is preceded by an possible Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-119

Clone 3-7

ATGGAAAAAGAAAAAAAATTAGGTCTTTTACCACTAACAATGCTTGTCATT
GGCTCTCTTATCGGTGGCGGAATCTTTGATTTAATGCAAAATATGAGTTCC
AGAGCCGGTTTGGTACCAATGCTTATTGCTTGGGTAATTACTGCTATCGGG
ATGGGAACTTTCGTTTTAAGTTTTCAAAATTTATCTGAAAAAAGGCCGGAC
CTAACAGCTGGAATCTTTAGTTACGCTAAAGAGGGGTTTGGAAACTTTATG
GGATTTAACTCTGCATGGGGTTATTGGTTATCAGCTTGGCTTGGAAATGTT
GCCTACGCTGCACTCTTATTCAGTTCACTCGGTTATTTCTTTAAATTCTTTG
GTAATGGAAATAATATCATCTCAATTATTGGAGCAAGTATAGTTATTTGGG
TTGTCCATTTCTTAATTTTAAGAGGTGTTAATACAGCTGCATTTATTAATAC
CGTAGTTACCTTTGCAAAATTAGTACCTGTTATTATTTTCTTAATTTCAGCG
TTATTAGCTTTCAAATTTAACATTTTTAGTCTTGATATCTGGGGAAATGGAT
TACATCAATCAATTTTCAACCAAGTCAATTCAACTATGAAAACCGCTGTTT
GGGTATTTATTGGTATTGAGGGCGCCGTTGTCTTCTCAGGTCGTGCTAAAA
AACACTCTGATATTGGTAAAGCAAGTATCCTAGCATTATTCACTATGATTT
CACTTTATGTATTGATTTCTGTTTTATCACTTGGTATCATGTCACGTCCAGA
ACTTGCAAACTTAAAAACACCAGCTATGGCTTACGTTCTAGAAAAAGCTGT
TGGTCACTGGGGTGCTATCTTAGTTAACCTTGGTGTTATCATTTCAGTATTT
GGCGCTATTCTTGCTTGGACTTTATTTGCAGCAGAATTACCATATCAAGCT
GCTAAAGAAGGTGCTTTTCCTAAATTTTTTGCAAAAGAAAATAAAAACAA
AGCTCCAATCAACTCACTCTTAGTCACTAATCTTTGTGTACAAGCATTCTTA
ATCACGTTCTTATTCACACAAAGTGCTTATCGTTTTGGTTTCGCATTAGCAT
CATCTGCTATCTTAATTCCTTATGCTTTTACAGCACTATATCAATTACAATT
CACACTCCGTGAGGATAAGTCAACTCCAGGACATCAAAAGAATTTAATTA
TCGGTATCCTCGCTACAATCTATGCTGTTTACCTTATCTACGCTGGTGGTTT
TGATTACTTACTTTTGACAATGATTGCTTATACTCTAGGTATGATTCTCTAT
ATTAAAATGAGAAAAGATGACAAGCTTGGCGTAATCATGGTCATAGCTGT
TTCCAGTGTGAAATTGTTATCC

MEKEKKLGLLPLTMLVIGSLIGGGIFDLMQNMSSRAGLVPMLIAWVITAIGMG
TFVLSFQNLSEKRPDLTAGIFSYAKEGFGNFMGFNSAWGYWLSAWLGNVAY
AALLFSSLGYFFKFFGNGNNIISIIGASIVIWVVHFLILRGVNTAAFINTVVTFAK
LVPVIIFLISALLAFKFNIFSLDIWGNGLHQSIFNQVNSTMKTAVWVFIGIEGAV

FIG. 1 CONT'D

VFSGRAKKHSDIGKASILALFTMISLYVLISVLSLGIMSRPELANLKTPAMAYV
LEKAVGHWGAILVNLGVIISVFGAILAWTLFAAELPYQAAKEGAFPKFFAKEN
KNKAPINSLLVTNLCVQAFLITFLFTQSAYRFGFALASSAILIPYAFTALYQLQF
TLREDKSTPGHQKNLIIGILATIYAVYLIYAGGFDYLLLTMIAYTLGMILYIKMR
KDDKLGVIMVIAVSSVKLLS

Sequence description:

A] Length: 1356 bp - 452 aa (partial sequence)
B] ATG start codon is preceded by an possible Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-120

Clone 3-8

ATGAAATTTGAAAAACGGCAGGTCTATTATGTTGTCATAACATTTGCTATT
TGCTATGCTATACAGGCTTATTGGGGAGCTGTTTCTAATATTTTAACTACGC
TTCATAAGGCAATATTTCCTTTTTTGATGGGAGCTGGAATTGCCTATATTAT
TAATATTGTAATGTCAGTCTATGAGCGATTATATATAAAGCTTTTTAAAGG
ATCTAGACTATTAATGGCAATCAAGCGTAGTGTTTCTATGATTTTATCCTAT
GCAACTTTTATTGGTTTAATTGTCTGGCTATTTTCAATTGTCATTCCAGATT
TGATTTCTAGTTTGAGTTCTTTATTGGTTATTGATACCGGAGCACTTGCTAA
ATTGGTTAATAATCTCAATGAAAATAAACAAATTTCTGAGGCTTTAAATTA
TATGGGAACAGATAAAGACTTAGTTTCTACTTTAAGTGGTTATAGCCAGCA
GATTTTGAAGCAAGTTTTATCTGTTTTAACAAATTTACTAACCTCAGTTTCC
TCTATTGCGGCAACACTTCTGAATGTTTTTGTTAGTTTTATTTTTTCAATTTA
CGTTTTGGCAAACAAGGAGCAGTTGGGACGTCAATTTAATTTGTTAATTGA
TACCTATTTAGGTTCAACAGGCAAAACATTCCATTACGTTCGTCATATCCTT
CATCAACGTTTCCATGGTTTTTTTGTAAGCCAAACTTTAGAAGCTATGATTT
TAGGAAGTTTGACGGTTATTGGTATGTTGATCTTCCAATTTCCTTATGCTTT
AACAGTTGGGGTTTTAGTTGCTTTTACAGCTCTAATACCGGTTGTGGGAGC
CTACATTGGTGTTACAATCGGTTTCATCTTAATTGCTACTGAATCGCTTACT
GAAGCATTCTTGTTTGTTCTTTTCTTGATCCTTTTACAACAATTTGAGGGAA
ATGTCATTTATCCGAAAGTTGTCGGTGGATCGATTGGACTGCCTTCTATGT
GGGTTTTAATGGCTATTACTATCGGAGGTGCTTTATGGGGGATCTTAGGCA

FIG. 1 CONT'D

TGTTACTTGCTGTTCCTGTTGCAGCTACTATCTATCAGATTGTAAAAGATCA
TATTATCAAGCGACAAACGCTTAGAAATCGTGCACGAACCTATCGTTAA

MKFEKRQVYYVVITFAICYAIQAYWGAVSNILTTLHKAIFPFLMGAGIAYIINI
VMSVYERLYIKLFKGSRLLMAIKRSVSMILSYATFIGLIVWLFSIVIPDLISSLSS
LLVIDTGALAKLVNNLNENKQISEALNYMGTDKDLVSTLSGYSQQILKQVLSV
LTNLLTSVSSIAATLLNVFVSFIFSIYVLANKEQLGRQFNLLIDTYLGSTGKTFH
YVRHILHQRFHGFFVSQTLEAMILGSLTVIGMLIFQFPYALTVGVLVAFTALIP
VVGAYIGVTIGFILIATESLTEAFLFVLFLILLQQFEGNVIYPKVVGGSIGLPSM
WVLMAITIGGALWGILGMLLAVPVAATIYQIVKDHIIKRQTLRNRARTYR*

Sequence description:

A] Length: 1134 bp - 378 aa (full-length gene)
B] ATG start codon is preceded by an typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-121

Identical to ID-68, as described in WO 00/06736

ID-122

Clone 3-16

GTGATTACAATTAAAAAGGAATCTGTTATCAAACTATTGAAGTATGCTTTT
GGCATTATAATGGGATTTATTATCTTAGCTATTGTAATAGGTGGGCTCCTA
TTTGCATACTACGTTAGTCGTTCTCCGAAATTAACCGATCAAGCTTTAAAA
TCCGTTAACTCTAGTTTGGTTTATGATGGTAATAATAAACTTATTGCCGATT
TAGGCTCAGAAAAGCGTGAAAGTGTTAGTGCGGATAGCATTCCACTAAAT
TTGGTTAACGCTATCACTTCTATAGAAGATAAACGTTTCTTTAAACATAGA
GGTGTCGATATTTATCGTATTTTAGGTGCAGCTTGGCATAACCTTGTTAGTA
GTAATACGCAAGGTGGTTCAACCCTTGATCAACAGTTGATTAAACTGGCTT
ACTTTTCTACCAATAAATCTGACCAAACGTTAAAACGTAAATCACAGGAA
GTTTGGCTTGCGCTTCAAATGGAGCGTAAATACACCAAAGAAGAAATTCTT
ACTTTCTATATTAATAAAGTTTATATGGGAAATGGGAATTATGGTATGAGA

FIG. 1 CONT'D

ACAACAGCTAAATCATACTTTGGTAAAGACCTAAAGGAATTATCTATTGCA
CAACTTGCTTTGCTCGCTGGTATTCCTCAAGCACCTACACAATATGACCCTT
ATAAAAACCCAGAATCTGCTCAAACAAGACGTAATACCGTTCTTCAGCAG
ATGTATCAAGATAAAAACATTTCTAAAAAGGAATACGACCAAGCTGTTGC
AACTCCAGTAACTGATGGCTTAAAAGAATTAAAGCAAAAATCTACTTATCC
AAAATATATGGATAACTACTTAAAACAAGTTATTAGTGAAGTTAAACAAA
AAACTGGTAAAGATATCTTTACTGCTGGGCTAAAAGTGTATACTAATATCA
ACACTGATGCACAAAAACAACTATATGACATCTACAACAGTGATACTTAC
ATCGCTTATCCAAACAATGAATTACAAATAGCATCTACCATCATGGATGCG
ACTAATGGTAAAGTCATTGCACAATTAGGCGGGCGTCATCAGAATGAAAA
TATTTCATTTGGGACAAATCAATCTGTCTTAACAGACCGCGATTGGGGTTC
TACAATGAAACCTATCTCAGCTTATGCACCTGCTATTGATAGTGGTGTCTA
TAATTCAACAGGTCAATCATTAAACGACTCAGTTTACTACTGGCCTGGTAC
TTCTACTCAACTATATGACTGGGATCGTCAATATATGGGTTGGATGAGTAT
GCAGACCGCTATTCAACAATCACGTAACGTCCCTGCTGTCAGAGCACTTGA
AGCCGCTGGATTAGACGAAGCAAAATCTTTCCTTGAAAAATTAGGCATAT
ACTATCCAGAAATG

MITIKKESVIKLLKYAFGIIMGFIILAIVIGGLLFAYYVSRSPKLTDQALKSVNSS
LVYDGNNKLIADLGSEKRESVSADSIPLNLVNAITSIEDKRFFKHRGVDIYRILG
AAWHNLVSSNTQGGSTLDQQLIKLAYFSTNKSDQTLKRKSQEVWLALQMER
KYTKEEILTFYINKVYMGNGNYGMRTTAKSYFGKDLKELSIAQLALLAGIPQA
PTQYDPYKNPESAQTRRNTVLQQMYQDKNISKKEYDQAVATPVTDGLKELK
QKSTYPKYMDNYLKQVISEVKQKTGKDIFTAGLKVYTNINTDAQKQLYDIYN
SDTYIAYPNNELQIASTIMDATNGKVIAQLGGRHQNENISFGTNQSVLTDRDW
GSTMKPISAYAPAIDSGVYNSTGQSLNDSVYYWPGTSTQLYDWDRQYMGWM
SMQTAIQQSRNVPAVRALEAAGLDEAKSFLEKLGIYYPEM

Sequence description:

A] Length: 1386 bp - 462 aa (partial sequence)
B] GTG start codon is preceded by an typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-123

Clone 3-17

FIG. 1CONT'D

ATGGCTAATGTATATGATTTAGCAAATGAATTAGAACGTGCTGTTCGTGCT
TTACCAGAATACCAAGCAGTTTTAACTGCAAAAGCAGCTATTGAAAATGA
TGCGGATGCACAAGTGCTTTGGCAAGACTTTTTGGCTACCCAATCAAAAGT
TCAAGAAATGATGCAATCTGGCCAAATGCCAAGTCAAGAAGAACAAGATG
AAATGTCTAAACTTGGGGAAAAAATTGAATCCAATGACCTTTTAAAAGTTT
ATTTTGACCAACAACAACGGTTGTCTGTCTATATGTCTGATATCGAAAAAA
TTGTCTTTGCACCCATGCAGGACTTGATGTAA

MANVYDLANELERAVRALPEYQAVLTAKAAIENDADAQVLWQDFLATQSK
VQEMMQSGQMPSQEEQDEMSKLGEKIESNDLLKVYFDQQQRLSVYMSDIEKI
VFAPMQDLM*

Sequence description:

A] Length: 336 bp - 112 aa (full length sequence)
B] ATG start codon is preceded by an typical Shine-Dalgarno sequence. No obvious potential leader peptide sequence.

ID-124

Clone 3-26

ATGGCAGAAATCACAGCTAAACTTGTAAAAGAATTGCGTGAAAAATCAGG
TGCAGGCGTTATGGACGCTAAAAAAGCATTAGTAGAAACTGATGGTGACC
TTGATAAAGCGATTGAATTACTTCGCGAAAAAGGTATGGCTAAAGCAGCT
AAAAAAGCAGACCGTGTTGCTGCTGAAGGTTTAACAGGTGTTTATGTTGAT
GGTAACGTTGCAGCAGTTATTGAAGTTAA

MAEITAKLVKELREKSGAGVMDAKKALVETDGDLDKAIELLREKGMAKAAK
KADRVAAEGLTGVYVDGNVAAVIEV

Sequence description:

A] Length: 230 bp - 76 aa (partial sequence)
B] ATG start codon is preceded by an typical Shine-Dalgarno sequence. No obvious potential leader peptide sequence.

FIG. 1CONT'D

ID-125

Clone 3-33

ATGATAAAAAACCTGTTATTAACAGGTTTTTTATCATTTAATGACGGAAAA
CTGGACACAAATTATTTTTCTTGTATAATTAAATATATTATTTCTTATCAGG
AGGTTATGATGACATTAGAGAAACGATTTAA

MIKNLLLTGFLSFNDGKLDTNYFSCIIKYIISYQEVMMTLEKRF

Sequence description:

A] Length: 134 bp - 44 aa (partial sequence)
B] ATG start codon is preceded by an typical Shine-Dalgarno sequence. Possible potential leader peptide sequence.

ID-126

Clone 3-41

ATGAAAAATAATAAAAATAATGGTTTTCTGAAAAATTCCTTTATTTACATA
TTATTGATTATTGCGGTTATTACAACCTTTCAATACTATTTAA

MKNNKNNGFLKNSFIYILLIIAVITTFQYYL

Sequence description:

A] Length: 94 bp - 31 aa (partial sequence)
B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence.

FIG. 1 CONT'D

ID-127

Clone 3-42

ATGTTAGATATTATCTTATCCGGAATTTCGCAAGGATTACTTTGGTCAATTA
TGGCAATTGGCGTGTTTATCACTTTTCGTATCTTAGACATAGCCGATCTCTC
TGCAGAAGGGGCTTTCCCTATGGGGGCTGCAGTTTGCGCCTTATGTATCGT
TAA

MLDIILSGISQGLLWSIMAIGVFITFRILDIADLSAEGAFPMGAAVCALCIV

Sequence description:

A] Length: 158 bp - 52 aa (partial sequence)
B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence.

ID-128

Clone 3-43

ATGGAAATGCCTAAAAGAAATGAATTACTCAATAAAGAAATTAAAATGAG
TATTGATAAACTTAGATATAAAGAACCAGAGAGTGAACATGACAAGCGAC
CTACTTTTTATTTGGTAGTACTTATACTTGTTACTGTAGCAGTTATATTGTC
GTTATTTAA

MEMPKRNELLNKEIKMSIDKLRYKEPESEHDKRPTFYLVVLILVTVAVILSLF

Sequence description:

A] Length: 161 bp - 53 aa (full-length gene)
B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential

FIG. 1 CONT'D

leader peptide sequence.

ID-129

Clone 3-44

GTGGTAAGTAAATTGAGTTTAACAACGATTTTTGCATTGCTATTTTCATCA
ATGCTAATTTACGCAACACCTCTTATCTTTACAAGTATTGGGGGAACCTTC
TCTGAACGTGGTGGTATCGTCAACGTTGGTTTAGAAGGAATTATGGTAATT
GGAGCTTTCTCAGGCGTTGTATTTAA

MVSKLSLTTIFALLFSSMLIYATPLIFTSIGGTFSERGGIVNVGLEGIMVIGAFSG
VVF

Sequence description:

A] Length: 179 bp - 59 aa (partial sequence)
B] GTG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence.

ID-130

Clone 3-46/47

ATGAGAATTATTGCAATAACTGAAAAGGTTATAAAAGAACTGTTTCGTGAT
AAAAGAACACTTGCTATGATGTTTTTAGCACCTATTTTAATTATGTTTTTGA
TGAATGTTATGTTTTCTGCGAATAGTAATACAAAAGTTAAGATTGGAACTA
TTAACGTTAACACGAAGGTCGTTTCAAATTTAGATAATATTAAGCATATTC
AAGTGAGATCATTTAAATTTAACTCATCTGCTAAAAAAGCACTCAAATCAA
ATAAAATTGATGCTCTTATTTCGGAGGACAATAAATCTTATACTGTCTTCT
ATGCGAATACAGATTCTTCAAAGACGACTTTAACAAGACAAGCTTTTAAA
ACCGCTGTTAATACAATGAACAGTAAGGAACTGATTTCGCAAGTTAAAATT
TTAGCTAATAAGAATCCGAAACTAGCACAATCCTTACAAACTCGCTCCAAA
TATATCAAAGAAAAATATAATTACGGAAATAAAAATACAGGCTTTTTTGC
AAAAATGATACCAATACTAATGGGATTTATGGTCTTCTTCTTGGTTTTT

FIG. 1 CONT'D

MRIIAITEKVIKELFRDKRTLAMMFLAPILIMFLMNVMFSANSNTKVKIGTINV
NTKVVSNLDNIKHIQVRSFKFNSSAKKALKSNKIDALISEDNKSYTVFYANTDS
SKTTLTRQAFKTAVNTMNSKELISQVKILANKNPKLAQSLQTRSKYIKEKYNY
GNKNTGFFAKMIPILMGFMVFFLVF

Sequence description:

A] Length: 558 bp - 186 aa (partial sequence)
B] ATG start codon is preceded by a possible Shine-Dalgarno sequence. Potential leader peptide sequence. C-terminus has yet to be determined.

ID-131

Clone 3-48

GTGATTATCGTTATGAGTAAACATCAAGAAATTTTGGAGTACCTAGAAAAT
TTAGCTGTTGGTAAGAGGGTTAGTGTACGCAGTATTTCAAATCATTTAA

MIIVMSKHQEILEYLENLAVGKRVSVRSISNHL

Sequence description:

A] Length: 100 bp - 33 aa (partial sequence)
B] GTG start codon is not preceded by a obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-132

Clone 2-c53

FIG. 1CONT'D

ATGTATAGAGAAATTACCGCTGTCGAACACGATCGCTTTGTGAGCGAATCC
AACCAAACAAACCTACTTCAATCTCTTAATTGGCCCAAAGTAAAAGACAA
CTGGGGTAGTCAATTACTTGGCTTTTTTGACGGTGAAACCCAAATTGCCAG
CGCTAGTATTCTCATCAAATCACTTCCTCTTGGCTTCTCCATGCTGTATATT
CCGCGTGGACCAATCATGGATTACTCCAATCTAGATATTGTAACTAAGGTC
CTTAAGGACCTTAAAGCTTTTGGCAAAAAACAAAGAGCTCTCTTTATCAAG
TGTGATCCTCTCATCTATTT

MYREITAVEHDRFVSESNQTNLLQSLNWPKVKDNWGSQLLGFFDGETQIASA
SILIKSLPLGFSMLYIPRGPIMDYSNLDIVTKVLKDLKAFGKKQRALFIKCDPLI
Y

Sequence description:

A] Length: 326 bp - 108 aa (partial sequence)
B] ATG start codon is preceded by an obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-133

Clone 2-c59

ATGGACAAGAAAAAAATCTTAGTAACGGGTATTGTGCCTAAAGAAGGTCT
AAGAAAGCTTATGGACCGATTTGATGTTACTTATTCAGAAGATCGCCCATT
TTCACGTGACTATGTGTTAGAGCATTTATCTGAATATGACGGATGGTTACT
CATGGGACAAAAAGGTGATAAAGAGATGATTGATGCAGGTGAAAACTTAC
AAATTATTTCTTT

MDKKKILVTGIVPKEGLRKLMDRFDVTYSEDRPFSRDYVLEHLSEYDGWLLM
GQKGDKEMIDAGENLQIIS

Sequence description:

A] Length: 215 bp - 71 aa (partial sequence)

FIG. 1CONT'D

B] ATG start codon is preceded by an obvious Shine-Dalgarno sequence. No obvious leader peptide sequence.

ID-134

Clone 2-c62

ATTTCGAAAGATGACTACCAAAATATTAGTTTTGGACAGGATCCAGAAGTT
GTTGATTATGCTGGTCTGTTTGAAAAACGCCGTCCAGTTTTAGAAAAAGCA
GTTAAAAATTTCTTGCAAGAAGAGAGAGCTACGAGAATGCTATCTGATTTC
TTGCAAGAAGAAAAATGGGTAACTGATTTTGCTGAATTTATGGCGATCAA
AGAACATTTTGGTAATAAGGCGCTTCAAGAATGGGATGACAAGGCTATTA
TACGCCGCGAAGAAGAAGCCTTAGCAGGATATCGTCAAAAGCTTAGTGAA
GTGATAAAATATCATGAAGTAACGCAATATTTCTTTTACAAACAATGGTTT
GAGTTAAAAGAATATGCTAATGATAAAGGGATTCAAATTATCGGTGATAT
GCCAATCTACGTTTCTGCCGATAGTGTAGAAGTTTGGACAATGCCTGAACT
GTTT

ISKDDYQNISFGQDPEVVDYAGLFEKRRPVLEKAVKNFLQEERATRMLSDFLQ
EEKWVTDFAEFMAIKEHFGNKALQEWDDKAIIRREEEALAGYRQKLSEVIKY
HEVTQYFFYKQWFELKEYANDKGIQIIGDMPIYVSADSVEVWTMPELF

A] Length: 459 bp - 153 aa (partial sequence)
B] More sequencing is required to determine the N- and C-termini
enzyme). - Streptococcus pneumoniae (63%)

ID-135

Identical to ID-108 described in WO 00/06736

Clone 2-c63

ID-136

Clone 2-c66

FIG. 1CONT'D

ATGGCAAAACAGAAAAATAACTGGCGCCGTGTTGGAGTTGGTGTCCTTAC
ACTTGCTTCAGTTGCGACTCTTGCTGCATGTGGAAGTAAATCAGCTTCCCA
GGATTCTAATGGAGCGATTAATTGGGCTATTCCAACAGAAATCAATACACT
AGATTTATCTAAAGTTACAGACACTTACTCAAATCTAGCTATTGGTAACTC
TAGTAGTAATTTCCTTCGCTTAGATAAAGATGGAAAGACAAGACCAGACTT
GGCTACTAAAGTTGATGTTTCAAAAGATGGCTTAACTTATACAGCTACATT
ACGTAAAGGCTTGAAGTGGTCAGATGGCAGTAAACTTACTGCAAAGGATT
TTGTTTATTCATGGCAACGTTTAGTTGATCCTAAAACAGCTTCACAATATG
CTTACCTTGCTGTTGAAGGGCATGTGCTTAATGCCGATAAAATCAACGAAG
GACAAGAGAAAGACTTGAATAAGCTAGGTGTTAAGGCAGAAGGCGATGA
CAAAGTTGTTATTACTTTATCTAGTCCGTCTCCGCAATTCATCTACTACCTT
GCATTCACTAACTTCATGCCACAAAAACAAGAAGTTGTTGAAAAATATGG
AAAAGATTACGCAACTACTTCAAAAAATACAGTTTACTCAGGACCATATA
CTGTTGAAGGTTGGAATGGTTCGAATGGTACTTTCACGCTGAAGAAAAAC
AAAAATTATTGGGACGCTAAAAATGTAAAAACAAAAGAAGTTCGCATCCA
GACTGTTAAAAAACCAGATACCGCCGTTCAAATGTATAAACGTGGTGAGT
TAGATGCAGCTAATATCTCAAATACTTCTGCTATTTATCAAGCTAATAAAA
ATAATAAAGATGTCACAGATGTTCTAGAAGCGACCACTGCCTATATGGAA
TATAATACTACTGGTTCTGTGAAAGGGCTTGATAATGTTAAGATTCGTCGC
GCCTTAAACTTAGCAACTAACCGTAAAGGAGTTGTTCAAGCAGCCGTTGAT
ACAGGCTCAAAACCGGCAATTGCTTTTGCACCTACTGGTTTAGCCAAAACA
CCAGATGGAACTGATTTGGCAAAATATGTTGCCCCAGGTTATGAATATAAT
AAAACTGAAGCAGCAAAACTCTTTAGACTA

MAKQKNNWRRVGVGVLTLASVATLAACGSKSASQDSNGAINWAIPTEINTLD
LSKVTDTYSNLAIGNSSSNFLRLDKDGKTRPDLATKVDVSKDGLTYTATLRKG
LKWSDGSKLTAKDFVYSWQRLVDPKTASQYAYLAVEGHVLNADKINEGQEK
DLNKLGVKAEGDDKVVITLSSPSPQFIYYLAFTNFMPQKQEVVEKYGKDYAT
TSKNTVYSGPYTVEGWNGSNGTFTLKKNKNYWDAKNVKTKEVRIQTVKKPD
TAVQMYKRGELDAANISNTSAIYQANKNNKDVTDVLEATTAYMEYNTTGSV
KGLDNVKIRRALNLATNRKGVVQAAVDTGSKPAIAFAPTGLAKTPDGTDLAK
YVAPGYEYNKTEAAKLFRL

Sequence description:

A] Length: 1143 bp - 381 aa (partial sequence)
B] Shine-Dalgarno sequence precedes ATG codon.
Possesses a potential leader peptide sequence.

FIG. 1 CONT'D

ID-137

Clone 2-c67

TTGAGAGTTTATGAAAATAAAGAAGAGTTGAAAAAAGAAATAAGTAAAAC
ATTTGAGAAATACATTATGGAATTTAATAA
TATTCCAGAGAATCTAAAAGATAAAAGAATTGATGAAGTTGATAGAACTC
CAGCAGAAAACCTTTCTTATCAGGTTGGCT
GGACCAACTTGGTTCTTAAATGGGAAGAAGATGAAAGAAAGGGACTTCAA
GTAAAAACACCATCGGATAAATTT

MRVYENKEELKKEISKTFEKYIMEFNNIPENLKDKRIDEVDRTPAENLSYQVG
WTNLVLKWEEDERKGLQVKTPSDKF

Sequence description

A] Length: 234 bp - 78 aa (partial sequence)
B] TTG start codon is preceded by a
potential Shine-Dalgarno sequence. No obvious
leader peptide sequence.

ID-138

Clone 2-c70

ATGTCAAAGTTTGATAGTCAGAAAATAATTACTCCGATTATGAAGTTTGTC
AATATGCGAGGGATTATTGCACTCAAAGATGGCATGCTAGCAATTTTACCA
CTAACAGTTGTTGGGAGTCTCTTTTTAATATTAGGGCAGCTTCCATTT

MSKFDSQKIITPIMKFVNMRGIIALKDGMLAILPLTVVGSLFLILGQLPF

Sequence description

A] Length: 150 bp - 50 aa (partial sequence)
B] ATG start codon is preceded by a potential
Shine-Dalgarno sequence. Possesses a potential

FIG. 1CONT'D

leader peptide sequence.

ID-139

Clone 2-c71

GAGACCACTTCATCAGTTAAACCAGCAGGAATTGACCGTATCAATCATACC
TCAACACCCCCGAAGAAAACTACCCCCAACATTGCAACGACGCATAGCTT
CAAAGATCGTTGTGATACTTTAGAAAGAATTCACAATGAAGACATTGATGT
TTGTTCTGGATTCATTTGTGGTATGGGAGAGAGCGATGAGGGGCTCATCAC
ATTAGCTTTCAGACTAAAAGAACTGAACCCCTATTCTATCCCTGTCAATTTT
TTACTTGCTGTTGAAGGAACACCTCTTGGAAAATATAACTATTTGACTCCC
ATTAAATGCTTAAAAATTATGGCCATGTTGCGTTTTGTTTTTCCTTTCAAGG
AATTAAGATTAAGTGCTGGACGGGAGGTCCATTTTGAGAATTTTGAATCAT
TAGTCACCTTACTTGTTGACTCAACTTTTTTGGGAAATTACCTAACAGAGG
GGGGTCGCAATCAACATACCGATATTGAATTCTTGGAAAAATTACAACTA
AATCATACTAAAAAGGAATTAATTT

ETTSSVKPAGIDRINHTSTPPKKTTPNIATTHSFKDRCDTLERIHNEDIDVCSGFI
CGMGESDEGLITLAFRLKELNPYSIPVNFLLAVEGTPLGKYNYLTPIKCLKIMA
MLRFVFPFKELRLSAGREVHFENFESLVTLLVDSTFLGNYLTEGGRNQHTDIEF
LEKLQLNHTKKELI

Sequence description:

A] Length: 535 bp - 178 aa (partial sequence)
B] N- and C-termini require verification

ID-140

Clone 2-c73

ATGCCGGTTTGGACTGCACAGTCTATTCCAAAGGCATTTTTAGAAAAGCAT
AATACTAAGGAAGGCACCTGGGCAAAACTAACCATTCTAAGTGGTTCTTTA
GTATTTTACCAGTTATCTCCTGATGGAGAGGAAATCTCGCGGCATATTTTT

FIG. 1 CONT'D

GATGCTAGTAGTGATATTCCTTTTGTTGATCCACAAGTCTGGCATAAAGTT
TCGCCGAATAGTCCAGACTTAAGTTGCTATCTAACTTTTTACTGCCAAAAA
GAAGATTACTTCCATAAAAAATATGGTCTCACGCGCACACATTCTGAGGTT
ATCGCCAGTGCACCTCTCTTATCTGAGAAGAGTAATATATTAGACCTTGGG
TGTGGTCAAGGGCGAAACTCACTTTATTTATCGCTGCTGGGACATCAAGTG
ACTTCTGTCGATTCAAACGGACAGAGCCTTGTAGCTTTAGAAAATATGGCA
TTAGAAGAAGAGCTTCCTTACAATATAAAAAGGTATGATATTAATACTACT
GCTATTGAAGGGCACTATGATTTTATTTTATCAACTGTGGTATTTATGTTTT
T

MPVWTAQSIPKAFLEKHNTKEGTWAKLTILSGSLVFYQLSPDGEEISRHIFDAS
SDIPFVDPQVWHKVSPNSPDLSCYLTFYCQKEDYFHKKYGLTRTHSEVIASAP
LLSEKSNILDLGCGQGRNSLYLSLLGHQVTSVDSNGQSLVALENMALEEELPY
NIKRYDINTTAIEGHYDFILSTVVFMF

Sequence description:

A] Length: 563 bp - 187 aa (partial sequence)
B] N- and C-termini require verification

ID-141

Clone 2c76

ATGACAAAGCAAATAATTGCCATTTGGGCTGAAGATGAAGACCATTTGAT
TGGAGTTAATGGCGGTTTACCATGGAGGCTTCCTAAAGAGTTACATCACTT
CAAAGAAACGACCATGGGGCAGGCTTTGCTTATGGGACGAAAGACCTTTG
ATGGAATGAACCGTCGTGTTTTACCTGGTAGAGAGACAATCATCTTAACAA
AAGATGAACAATTCCAAGCAGATGGAGTGACAGTCCTAAATAGTGTTGAA
CAAGTTATAAAATGGTTTCAGGAACATAATAAGACCTTATTTATTGTAGGT
GGTGCAAGTATTTATAAAGCATTTCTGCCTTATTGTGAAGCAATCATAAAA
ACTAAAGTTCATGGAAAATTCAAAGGTGATACCTATTTTCCTGATGTTAAT
CTATCTGAGTTT

MTKQIIAIWAEDEDHLIGVNGGLPWRLPKELHHFKETTMGQALLMGRKTFDG
MNRRVLPGRETIILTKDEQFQADGVTVLNSVEQVIKWFQEHNKTLFIVGGASI
YKAFLPYCEAIIKTKVHGKFKGDTYFPDVNLSEF

FIG. 1 CONT'D

Sequence description:

A] Length: 417 bp - 139 aa (partial sequence)
B] ATG start codon is preceded by a Shine-Dalgarno sequence. No leader peptide sequence

ID-142

Clone 2-c78

TTGTGGCCAAACTGTGCCCCGCTTATTAATAGCACTTTGTTCACCATTGAA
GATATCTTAACATCAGGTGCTCATAGCAACCCTATTTTAATGGGGGTTATA
CTTGGCGGGACAATTGTAGTAGTGGCGACAGCACCACTTTCTTCTATGGCA
TTGACAGCTATGCTAGGATTAACCGGAATGCCTATGGCTATAGGAGCCTTG
TCTGTCTTTGGTTCGTCATTTATGAATGGTGTACTTTTCCATAAATTAAAAC
TTGGAAGTCGTAAAGATAATATAGCTTTTGCTGTTGAGCCTCTAACTCAAG
CTGACGTGACTTCAGCTAACCCTATTCCAATCTATGTCACTAATTTTGTTGG
TGGTGCAGCTTGTGGTATTTTAATTGCCTTGATGAAATTAGTTAATGATACT
CCTGGAACAGCGACACCAATTGCAGGATTTGCTGTCATGTTTGCCTATAAC
CCAATGATAAAAGTACTAATAACCGCTCTAGGTTGTATTATCCTATCTTTA
CTAGCAGGCTATTTTGGAGGCATTGTTTTT

MWPNCAPLINSTLFTIEDILTSGAHSNPILMGVILGGTIVVVATAPLSSMALTA
MLGLTGMPMAIGALSVFGSSFMNGVLFHKLKLGSRKDNIAFAVEPLTQADVT
SANPIPIYVTNFVGGAACGILIALMKLVNDTPGTATPIAGFAVMFAYNPMIKVL
ITALGCIILSLLAGYFGGIVF

Sequence description:

A] Length: 540 bp - 180 aa (partial sequence)
B] N- and C-termini have yet to be elucidated

ID-143

FIG. 1 CONT'D

Clone 2-c80

ATGTTTTTAAGTATAATGGCAGGTGTCATAGCATTTGTCCTGACAGTTATT
GCCATTCCACGCTTCATTAAGTTTTACCAATTGAAGAAAATTGGCGGGCAA
CAAATGCATGAAGATGTCAAACAACATCTAGCCAAAGCAGGTACGCCGAC
AATGGGAGGAACGGTATTTT

MFLSIMAGVIAFVLTVIAIPRFIKFYQLKKIGGQQMHEDVKQHLAKAGTPTMG
GTVF

Sequence description:

A] Length: 172 bp - 57 aa (partial sequence)
B] Shine Dalgarno sequence precedes 'ATG' start codon. Possesses a potential leader peptide sequence.

ID-144

Clone 3-83

ATGAAACCATATTTATCTTTTATTGGTAGAACGTTATTATACTTCGGTATTT
TATTGTTACTAATTTACTTTTTTGCATACCTTGGTCGCGGACAAGGCAGTTT
TATTTATAA

MKPYLSFIGRTLLYFGILLLLIYFFAYLGRGQGSFIY

Sequence description:

A] Length: 113 bp - 37 aa (partial sequence)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.
This orf is not in frame with nuc

FIG. 1CONT'D

ID-145

Clone 3-86

ATGTCATATTTTAGAAATTACTGGTATCGTTTTGGAGCAATTTTATTTATTA
TTTTAGCAGTAATATTGCTTGTTTTTAGACCTGACTGGTCAATGCTTCACTA
TCTATTGTATTTTTACTTTATGGCACTTCTAGCGCATCAATTTGAAGAATAT
CAGTTTCCCGGTGGGGCATCACCTATCATTAACTATGTTGTTTATGATGAA
GAAGAGCTGATGGATTGTTTTCCAGGCAATACTCAGTCTATTATGTTGGTT
AATACTATTGCTTGGTTGCTTTACATTGCTAGTATTGCTTTTCCTCAAGCTT
ATTGGCTTGGATTAGGAGTCATGTTCTTTAGTCTAACGCAGCTCTTGGGTC
ATGGTTTTCAGATGAATATTAAACTTAAAACTTGGTATAATCCTGGTCTAG
CAACGACAGTATTTCTCCTAGTACCAATAGCTTGCGCATACATCTATCAAG
CTAGTGCAGAAGGAATGCTCACTTGGGGAGATTGGCTAGGTGGTTTTATCA
TGTTGATTGTCTGTGTACTAACTAGCATTATTGCACCTGTACAGCTATTGAA
GGATAAGGAGACCAATTATATTATTAGTCCTTGGCAAATGGACCGTTTTCA
TAAGGTCGTTAATTTTGTAAGGATAAAAAAATAA

MSYFRNYWYRFGAILFIILAVILLVFRPDWSMLHYLLYFYFMALLAHQFEEYQ
FPGGASPIINYVVYDEEELMDCFPGNTQSIMLVNTIAWLLYIASIAFPQAYWLG
LGVMFFSLTQLLGHGFQMNIKLKTWYNPGLATTVFLLVPIACAYIYQASAEG
MLTWGDWLGGFIMLIVCVLTSIIAPVQLLKDKETNYIISPWQMDRFHKVVNFV
RIKK*

Sequence description:

A] Length: 651 bp - 219 aa (full length gene)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.

ID-146

Clone 3-c88

FIG. 1 CONT'D

ATGCCACTTACAGCACTTGAAATTAAAGATAAAACATTTTCATCAAAATTT
CGCGGTTATAGCGAAGAAGAAGTT

MPLTALEIKDKTFSSKFRGYSEEEV

Sequence description:

A] Length: 75 bp - 25 aa (partial sequence)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. No leader peptide

ID-147

Clone 3-90

ATGTCACTTTTTCAAGAAAAAATTGCTTACAATTGCGCTAAAAAGGAAGCG
CTTTATAAAGAGAGTTTAGGACGCTACGCCTTGAGATCAATGCTAGCAGG
GGCTTATTTGACAATGAGTACTGCTGCCGGTATCGTCGCAGCTGATACTAT
TGGTAAAATTTCTCCTGCTCTATCAGGTTTTGTATTTGCTTTCATCTTTAGTT
TTGGACTTATTTATGTTTTAATATTTAATGGTGAATTGGCGACATCTAATAT
GCTTTATCTCACTGCAGGAGCCTATAATAAAAATATCTCTTGGAAAAAAGC
CATAACAATTTTAATTTATTGTACTTTTTTCAACCTCGTTGGTGCTTGTATA
TTAGCTTGGTTGTTTAA

MSLFQEKIAYNCAKKEALYKESLGRYALRSMLAGAYLTMSTAAGIVAADTIG
KISPALSGFVFAFIFSFGLIYVLIFNGELATSNMLYLTAGAYNKNISWKKAITILI
YCTFFNLVGACILAWLF

Sequence description

A] Length: 406 bp - 125 aa (partial sequence)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possible leader peptide

FIG. 1 CONT'D

ID-148

Clone 3-92

AAGTTACAAGCGACTGAAGTTAAGAGCGTTCCGGTAGCACAACCAGCTTC
AACAACAAATGCAGTAGCTGCACATCCTGAAAATGCAGGGCTCCAACCTC
ATGTTGCAGCTTATAAAGAAAAAGTAGCGTCAACTTATGGAGTTAATGAA
TTCAGTACATACCGTGCGGGAGATCCAGGTGATCATGGTAAAGGTTTAGC
AGTTGACTTTATTGTAGGTAAAAACCAAGCACTTGGTAATGAAGTTGCACA
GTACTCTACACAAAATATGGCAGCAAATAACATTTCATATGTTATCTGGCA
ACAAAAGTTTTATTCAAATACAAATAGTATTTATGGACCTGCTAATACTTG
GAATGCAATGCCAGATCGTGGTGGCGTTACTGCCAACCACTATGACCACGT
TCACGTATCATTTAA

KLQATEVKSVPVAQPASTTNAVAAHPENAGLQPHVAAYKEKVASTYGVNEF
STYRAGDPGDHGKGLAVDFIVGKNQALGNEVAQYSTQNMAANNISYVIWQQ
KFYSNTNSIYGPANTWNAMPDRGGVTANHYDHVHVSF

Sequence description

A] Length: 419 bp - 139 aa (partial sequence)
B] N- and C-termini have yet to be determined

ID-149

Clone 3-94

ATGATTCCAGTAGTTATTGAACAAACAAGTCGTGGTGAACGTTCTTATGAT
ATTTACTCACGTCTTTTAAAAGATCGTATTATTATGTTGACAGGCCAAGTT
GAGGATAATATGGCCAATAGTATCATTGCACAGTTATTGTTTCTCGATGCA
CAAGATAATACAAAGGATATTTACCTTTATGTCAATACACCAGGTGGTTCA
GTATCGGCTGGACTTGCTATTGTGGACACCATGAACTTCATTAAATCGGAC
GTACAGACGATTGTTATGGGGATGGCTGCTTCGATGGGAACCATTATTGCT
TCAAGTGGTGCTAAAGGAAAACGTTTTATGTTACCGAATGCAGAATATATG

FIG. 1 CONT'D

ATCCACCAACCAATGGGCGGAACAGGCGGAGGTACACAGCAATCTGATAT
GGCTATCGCTGCTGAGCATCTTTTAAAAACGCGTCATACTTTAGAAAAAAT
CTTAGCTGATAATTCTGGTCAATCTATTGAAAAAGTCCATGATGATGCAGA
GCGTGATCGTTGGATGAGTGCTCAAGAACACTTGATTATGGCTTTATTGAT
GCTATTATGGAAAATAATAATTTACAATAATAGATTTAAAAGAGTTGAGTT
TACCAACTCTTTTTTTATTTGTTGGAATTATGTTATAATCTTAGTAATTACA
GATATGACGCAGAAAGGAAAAAATTATTGA

MIPVVIEQTSRGERSYDIYSRLLKDRIIMLTGQVEDNMANSIIAQLLFLDAQDN
TKDIYLYVNTPGGSVSAGLAIVDTMNFIKSDVQTIVMGMAASMGTIIASSGAK
GKRFMLPNAEYMIHQPMGGTGGGTQQSDMAIAAEHLLKTRHTLEKILADNSG
QSIEKVHDDAERDRWMSAQEHLIMALLMLLWKIIIYNNRFKRVEFTNSFFICW
NYVIILVITDMTQKGKNY*

Sequence description

A] Length: 693 bp - 231 aa (full length gene)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. No leader peptide. Significantly, it would appear to have a very hydrophobic C-terminus.

ID-150

Clone 2-c86

ATGAAACCAAAAaTTATTGGTGTACTTGGTCTAGGAATATTTGGACAAACA
CTCGCACAAGAACTAAGTAACTTTGAACAAGATGTTATTGCTATTGACAGC
AATCCTGAAAATGTACAAGCTGTCGCCGAAGT
TGTTACAAAAGCAGCTATCGGAGACATTACTGATTTAGCTTTCCTAAAACA
CATCGGGATCAGTGACTGTGATACTGTTATTATTGCTACAGGAAACAGTTT
AGAGAGCTCAGTATTGGCCGTAATGCACTGTAAAAAGTTAGGCGTCCCAC
AAGTTATTGCTAAAGCTCGAAACCTTGTATACGAAGAAGTACTTTATGAAA
TTGGTGCTGATTTGGTTATCTCTCCGGAGCGAGAATCTGGGCAAAATGTTG
CTGCAAACCTCATGAGAAATAAAATTACAGATGTCTTCCAGATTGAATCTG
ATATTTCTGTCATTGAATTT

MKPKIIGVLGLGIFGQTLAQELSNFEQDVIAIDSNPENVQAVAEVVTKAAIGDI
TDLAFLKHIGISDCDTVIIATGNSLE

FIG. 1CONT'D

SSVLAVMHCKKLGVPQVIAKARNLVYEEVLYEIGADLVISPERESGQNVAAN
LMRNKITDVFQIESDISVIEF

Sequence description:

A] Length: 459 bp - 153 aa (partial sequence)
B] Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a potential leader peptide sequence.
This orf is not in frame with nuc

ID-151

Clone 2-c88

GTGCGTTATAGTAAAGAGATTATTCAGTTAGCTATACCAGCTATGATTGAA
AATATCTTACAAATGCTCATGGGAGTAGTTGATAATTATCTAGTGGCTCAG
TTAGGTGTTGTAGCAGTATCAGGTGTTTCAGTTGCTAATAATATAATTACT
ATTTATCAAGCTATTTTTATAGCTTTAGGGGCGAGTATAGCAAGTCTATTG
GCCAAGTCGTTAGCAGGTAGTGAGAAGGATGATGCAATTTCAGTATGTTCT
CAAGCCATTTTTCTAACATCACTGATAGGGGCAGTATTAGGAATTATCTCG
ATTGTTTTTGGACAAACTTTCTTT

MRYSKEIIQLAIPAMIENILQMLMGVVDNYLVAQLGVVAVSGVSVANNIITIY
QAIFIALGASIASLLAKSLAGSEKDDAISVCSQAIFLTSLIGAVLGIISIVFGQTFF

Sequence description

A] Length: 330 bp - 110 aa (partial sequence)
B] Putative GTG start codon is preceded by a typical Shine-Dalgarno sequence. May have a leader peptide

ID-152

FIG. 1 CONT'D

Clone 2-c92

TTGATTAACAAGTATTCGTGCTTTTTGAAGAGGATTCTCCATAATAATACT
CCTTTAATAGTTATCGTGAGAAGTATTTTAAAGAAAAACCGCCAAGGTAG
AGCGACATTTCTGCCTTTAACTACAATAAAACCAAGAGAATTAGCACAAC
ATTATCTCTCAAAATTACAAAGTTCTCAAGGGTTTTTAGGAATAGCTAGTG
AATTGGTAACCTATGATCAACGCTTGTCAAACATTTTT

MINKYSCFLKRILHNNTPLIVIVRSILKKNRQGRATFLPLTTIKPRELAQHYLSK
LQSSQGFLGIASELVTYDQRLSNIF

Sequence description

A] Length: 240 bp - 80 aa (partial sequence)
B] No obvious Shine Dalgarno sequence precedes the Putative TTG start codon

ID-153

Clone 2-c94

TTGTTGACTCACAAAAATATATTATTAACCATTATATTTGGATTATTTATGA
TTATATTATCAGCATGTGGTATGTCTAATAAGGAAATGGCTGGTATTGATA
ATTGGGAACATTATCAAAAGGAAAAGAAAATTACTATTGGATTTGATAAT
ACTTTTGTTCCTATGGGATTTGAAAGTCGTTCTGGTGACTATACCGGCTTTG
ATATTGATTTAGCTAATGCTGTTTTTAAAGAATACGGTATTTCAGTGAAAT
GGCAGCCTATTAACTGGGATATGAAAGAAACTGAACTTAATAATGGTAAT
ATAGACCTTATTTGGAATGGTTATTCAAAAACGGCAGAACGTGCTAAAAA
AGTCGCTTTTACAAACCCATATATGAATAATCATCAAGTAATTGTTACTAA
AACTTCATCACATATTAATAGTATTAAGGATATGAAGGGGAAAAAACTAG
GAGCCCAGTCGGGTTCATCTGGTTTTGATGCTTTTAACGCTAAACCTGATA
TTTTAAAAAAGTTTGTAAAAGGAAAAGAAGCAGTTCAATACGATACTTTC
ACTCAGGCTTTGATTGATTTAAAAAATAACCGTATTGATGGTCTTTTGATT
GATGAAGTTTATGCTAACTATTATTTAAAGCAAGAAGGAA

FIG. 1CONT'D

MLTHKNILLTIIFGLFMIILSACGMSNKEMAGIDNWEHYQKEKKITIGFDNTFV
PMGFESRSGDYTGFDIDLANAVFKEYGISVKWQPINWDMKETELNNGNIDLI
WNGYSKTAERAKKVAFTNPYMNNHQVIVTKTSSHINSIKDMKGKKLGAQSG
SSGFDAFNAKPDILKKFVKGKEAVQYDTFTQALIDLKNNRIDGLLIDEVYANY
YLKQEG

Sequence description

A] Length: 649 bp - 216 aa (partial sequence)
B] TTG start codon is preceded by a possible typical Shine-Dalgarno sequence. Has a leader peptide

ID-154

Clone 2-c100

ATGAAAATTTGGAAAAAAATAACCTTAATGTTTTCTGCAATTATTTTAACA
ACAGTAATTGCATTGGGAGTCTATGTTGCCTCAGCTTATAATTTTTCGACTA
ATGAATTGTCTAAGACTTTT

MKIWKKITLMFSAIILTTVIALGVYVASAYNFSTNELSKTF

Sequence description

A] Length: 123 bp - 41 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Has a typical leader peptide

ID-155

Clone 2-c1

FIG. 1CONT'D

ATGAAAAAACAAAGACTATTACTGCTTTTTGGAGGCTTATTAATAATGATA
ATGATGACAGCATGTAAGGATTCAAAAATCCCAGAAAACCGCACGAAAAA
GGAATACCAGGCAGAACAGAATTTTAAGTCATACTTTAAATATATATCAG
ATAAAAATAACTATTTAGATAATATAAAAGTTTATTACTTTTCTATAAGTA
TTTCTAAAGATGTACAAGATAAAGTCAGTGAAACAACAACTTGTTCATATA
GACTAGAAAAGCAAAAGAATCAAGAGTTCATTGGTAATTTTGAACATGAA
GTTAGTGAATCTAGTCAATATTCAACCGAAGTTAAAAATCAAATACAGTAT
CCAATCCAGTATAAAGATAATTCAATTCGTTTTACTGAAAAAACACCGTCA
GAACGTTATGATGAGTTTGTTTTTAGTTCATTTGATTCTTCATTATTAAAAA
AATATAAAATATATGATTACTTACTAAAACATCCCGAAACTGAATTAAAA
GGTGTTTCCTATAAGATTCCTATAAATTCTGAAATTGTAGCCCCTTTTATAA
ATCAATTAAATATAAAAAATCCTAAAAAATCATCTATTTCGGTTACAAAAA
CGGAAAGTAAAGAATATTATTATACAATCAGTATTGATACTGATTCTGAGA
TATATTCTATATTCGAAGGTATTCAT

MKKQRLLLLFGGLLIMIMMTACKDSKIPENRTKKEYQAEQNFKSYFKYISDKN
NYLDNIKVYYFSISISKDVQDKVSETTTCSYRLEKQKNQEFIGNFEHEVSESSQ
YSTEVKNQIQYPIQYKDNSIRFTEKTPSERYDEFVFSSFDSSLLKKYKIYDYLLK
HPETELKGVSYKIPINSEIVAPFINQLNIKNPKKSSISVTKTESKEYYYTISIDTDS
EIYSIFEGIH

Sequence description

A] Length: 687 bp - 229 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Has a typical leader peptide. C-terminus has yet to be verified

ID-156

Clone 2-c5

ATGACATTTGACACCATTGATCAATTAGCGGTTAATACAGTCCGCACGCTT
TCTATTGATGCTATCCAAGCAGCAAATTCTGGGCACCCAGGTCTTCCTATG
GGAGCTGCGCCTATGGCTTATGTGCTTTGGAATAAATTCTTAAATGTAAAC
CCAAAAACAAGTCGCAATTGGACAAACCGTGACCGTTTTGTACTTTCAGCT

FIG. 1 CONT'D

GGGCATGGTTCAGCTCTTCTTTATAGCCTACTTCATTTAGCTGGCTATGATT
TATCAATTGATGATTT

MTFDTIDQLAVNTVRTLSIDAIQAANSGHPGLPMGAAPMAYVLWNKFLNVNP
KTSRNWTNRDRFVLSAGHGSALLYSLLHLAGYDLSIDD

Sequence description

A] Length: 272 bp - 90 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. No obvious leader peptide

ID-157

Clone 2-c8

ATGAGAACACTATTTAGAATGATATTTGCTATTCCAAAGTTTATCTTTAGA
TTGATTTGGAATATCATTTGGGGAATATTCAAGACAGTTCTTGTTATTGCG
ATTATTTTATTTGGCTTGTATTACTATGCGAATCACAGTCAATCAGAATTTG
CTAATCAACTTAGTGACATTATTCAGACAGGAAAAACATTTTT

MRTLFRMIFAIPKFIFRLIWNIIWGIFKTVLVIAIILFGLYYYANHSQSEFANQLS
DIIQTGKTF

Sequence description

A] Length: 197 bp - 65 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Possesses a leader peptide

ID-158

FIG. 1CONT'D

Clone 2-c9

ATGTCAAAAAAAATAATATTAGGAATTTTATCTCTTTTATCTGTCGTTACTT
TGGTGGCGTGTGGTTCATCAGACAAACAGCTACAAGATAAAGTTGAGAAA
AAAGGGAAGTTAGTTTTAGCGGTGAGTCCAGATTATGCTCCCTTTGAGTTT

MSKKIILGILSLLSVVTLVACGSSDKQLQDKVEKKGKLVLAVSPDYAPFEF

Sequence description

A] Length: 153 bp - 51 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Possesses a leader peptide (not in frame with nuc)

ID-159

Clone 2-c10

ATGAAAAATCAAAGACTATTACTGCTTTTTGGAGGCTTATTAATAATGATA
ATGATGACAGCATGTAAGGATTCAAAAATCCCAGAAAACCGCACGAAAAA
GGAATACCAGGCAGAACAGAATTTTAAGTCATACTTT

MKNQRLLLLFGGLLIMIMMTACKDSKIPENRTKKEYQAEQNFKSYF

Sequence description

A] Length: 139 bp - 46 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Possesses a leader peptide

FIG. 1CONT'D

ID-160

Clone 2-c11

ATGATTGGAAAATTATATTATAGCTATAGAAAGTCACGCTTATTAAGAAGT
ATTTTATGGCTTATTTTAATTGTTGGTGTATATATGTTAGGACAACGTGTTT
TATTATCCACTGTTCCTTTATCACATCAAGAGATAAAACTAGCAGTAGATC
AACATTTACTCAATAACTTTTCAGCAGTAAGTGGTGGGAGTTTTAATAAAT
TAAATGTTTTCACACTGGGGTTGAGTCCATGGATGTCAAGTATGATTATTT
GGAGATTCGTTTCCTTATTTTCGTGGGCAAAAAATGCAACGAAGCGAAAA
GCAGAAGTAGCTCAATATACTTTAATGCTTACTATCTCAGTTATACAAGCA
TATGGTGTTTCAGGAAATCAATTTATAAAAAGCTCTTTATTAGGTTCTTATA
GTGATATTGTTTTT

MIGKLYYSYRKSRLLRSILWLILIVGVYMLGQRVLLSTVPLSHQEIKLAVDQHL
LNNFSAVSGGSFNKLNVFTLGLSPWMSSMIIWRFVSLFSWAKNATKRKAEVA
QYTLMLTISVIQAYGVSGNQFIKSSLLGSYSDIVF

Sequence description

A] Length: 423 bp - 141 aa (partial sequence)
B] ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Possesses a leader peptide

ID-161

Clone 2-c13

ATGAAAGGTCTATTGGATTTTTTAGTTAATATTGCCAGAACGCCAGCTATT
TTAGTCGCCTTGATAGCCATTATCGGTTTAGTACTGCAGAAAAAAGGTGTT
CCTGATATTGTAAAAGGTGGAATAAAAACATTTGTTGGCTTCTTAGTGGTT
TCTGAAGGTGCAGGGATAGTCCAAAATTCCTTGAATCCATTTGGAAAAATG
TTTGAACATGCTTTTCATTTGGTGGGGGTAGTTCCTAATAATGAAGCCATT
GTAGCAGTAGCTCTTACGAAGTATGGCTCAGCAACTGCTTTGATTATGTTA
GCGGGAATGATTTTTAATATTTTAATTGCTCGTTTTACAAAA

FIG. 1CONT'D

MKGLLDFLVNIARTPAILVALIAIIGLVLQKKGVPDIVKGGIKTFVGFLVVSEG
AGIVQNSLNPFGKMFEHAFHLVGVVPNNEAIVAVALTKYGSATALIMLAGMI
FNILIARFTK

Sequence description

A] Length: 348 bp - 116 aa (partial sequence)
B] ATG start codon is preceded by a potential Shine-Dalgarno sequence. Possible leader peptide

ID-162

Clone 2-c21

TTGGTTGGTAAGCCCCAATTACTATTTTTAGATGAACCTACTTCCGGAATG
GATACTTCCACACGTCAACGATTTTGGAAGCTGGTTGCGACACTAAAAAA
AGAAGGTGACACAATTGTCTATTCTAGTCATTATATCGAAGAGGTAGAAC
ATACAGCTGATAGGATTTTAGTACTTCATAAAGGAAAGTTATTACGCGATA
CAACCCCCTTTGCCATGAAGCAAGAAAAAACCGAAAAGTTATTCACCGTT
CCGCTTAGTTATCAAAAATTATTACCTACCTATTTGATTACAGAGTGTGAA
GCCAAGAGTGATAGTATAACGTTTGTTACTGGGGAGGCTGAAACTGTATG
GAAAATACTGGCAGATAATGGTTGTCCTATTGAAGCTATTGAGATGACCA
ATAGAACTTTGTTAAATCGTATTTTTGAGACTACTAAGGAGGTAAAACATG
AGAATCTTTA

MVGKPQLLFLDEPTSGMDTSTRQRFWKLVATLKKEGDTIVYSSHYIEEVEHTA
DRILVLHKGKLLRDTTPFAMKQEKTEKLFTVPLSYQKLLPTYLITECEAKSDSI
TFVTGEAETVWKILADNGCPIEAIEMTNRTLLNRIFETTKEVKHENL

Sequence description

A] Length: 462 bp - 155 aa (partial sequence)
B] B] Putative TTG start codon is not preceded by an obvious Shine-Dalgarno sequence. No obvious leader peptide. N- and C- termini require further

FIG. 1CONT'D

examination.

ID-163

Clone 2-c25

TTGAAAAAATCCAAGAGAAGCCGTAAGGCAGTGACAACAAGTGGTGAGA
AGACTTTACTTGAGGATTTGGCAAAAATGAATTTCCTAGACGAAGTCATTA
ATGTTATGGTTTTATATACCTTGAATAAGACAAAATCTGCTAACTTAAATA
AGGCCTATATCATGAAAGTTGCTAATGATTTTGCCTTTCAGAATGTTATGA
CGGCCGAAGATGCTGTGCTTAAAATTCGTGATTTTTCAGATCAAAAAGTAA
GGACTAAAACAGAAACGAAGAAGAAACAATCGAATGTTCCTGAATGGAGT
AATCCTGATTATAAAGATGAGGTTAGCCCAGAAAAAGAAATTGAATTAGA
ACAGTTT

MKKSKRSRKAVTTSGEKTLLEDLAKMNFLDEVINVMVLYTLNKTKSANLNK
AYIMKVANDFAFQNVMTAEDAVLKIRDFSDQKVRTKTETKKKQSNVPEWSN
PDYKDEVSPEKEIELEQF

Sequence description

A] Length:360 bp - 120 aa (partial sequence)
B] N- and C- termini require verification.

ID-164

Clone 2-c28

ATGACGAATCATATTACTAAACTGATAGAAAATAGCGGAAAAAAAATTGAC
AGAAATTAGCGAAGCTACAGATATAGCCTATCCTACACTTTCTGGATACAA
TCAAGGAATCCGCAAACCTAAAAAAAGATAATGCTGAAAAATTGGCAAAAT
ACTTTAATGTTTCCGTCGCTTACATTATGGGACTTGATAGCAACCCACATG
CTCCATCAAATCTT

MTNHITKLIENSGKKLTEISEATDIAYPTLSGYNQGIRKPKKDNAEKLAKYFNV
SVAYIMGLDSNPHAPSNL

FIG. 1CONT'D

Sequence description

A] Length:218 bp - 72 aa (partial sequence)
B] ATG start codon is preceded by an obvious Shine Dalgarno sequence. No obvious leader peptide.

ID-165

Clone 2-c29

TTGATGAAAAGGAATAAACATTTACCGTTAACAGAAACTACCTATTATATT
TTATTAGCTTTGTTTGAGGAAGCGCATGGCTATGCTATTATGAAAAAAGTT
GAAGAAATGAGTGGCGGTGATGTTAGAATAGCCGCAGGGACAATGTACGG
TGCCATTGAAAATTTACTTAAACAAAAATGGATAAAGTCTATCTCAAGTGA
CGATAGAAGAAGAAAAGTTTATATTATTACTGAGACAGGAAAAGAAATAG
TAGAACTTGAAACGAATCGATTAAGAAAGTTACTTAATACTGCTAATCAGT
TGGGTTTTGGAGGAGATGGTTATGATAAAGTTT

MMKRNKHLPLTETTYYILLALFEEAHGYAIMKKVEEMSGGDVRIAAGTMYG
AIENLLKQKWIKSISSDDRRRKVYIITETGKEIVELETNRLRKLLNTANQLGFG
GDGYDKV

Sequence description

A] Length:337 bp - 112 aa (partial sequence)
B] TTG start codon is preceded by an obvious Shine Dalgarno sequence. Actual start codon may ATG that comes immediately after the TTG. Potential leader peptide.

ID-166

FIG. 1 CONT'D

Clone 2-c35

CCCATTACTGGTGAGTTAATAGCTGAGAAATTAGGAGTACCAAGAGCAGC
ACTAAGGTCTGATTTGCGGGTTTTAAGTATGCTAGGTATCATAGATGCAAA
ACCTAAGGTTGGTTATTTTTATTTAGGACAGTATCATGCTTCAATAGGGAC
AAGTCATTTTGAAAAGATGACAGTTTCAGAAATTATGGGGATCCTTCTGAC
AGTTCATCAAAAAGATTCAGTTTATGATGTTATTGTACATATTTTTATGGA
AGATGCTGGTTGTGCTTTTATCTTGGATGATGATGATTTTCTCTGTGGAGTC
GTGTCACGTAAAGATTTACTAAAAACCAGTATTGGCGGAGGAGATCTTTCT
AAAATGCCAATAGGAATGGTGATGACACGTATGCCACACGTGACAACTGT
TTTAGAAAATGAAAGTCTTTTTGCGGCAGCTGATAAATTAGTGAGCAGAA
AAGTGGATAGTCTCCCTGTCGTTCGTCATGATAAGCAATATCCCGAAAAAT
TTA

PITGELIAEKLGVPRAALRSDLRVLSMLGIIDAKPKVGYFYLGQYHASIGTSHF
EKMTVSEIMGILLTVHQKDSVYDVIVHIFMEDAGCAFILDDDDFLCGVVSRKD
LLKTSIGGGDLSKMPIGMVMTRMPHVTTVLENESLFAAADKLVSRKVDSLPV
VRHDKQYPEKF

Sequence description

A] Length:511 bp - 170 aa (partial sequence)
B] N- and C-termini to be determined

ID-167

Clone 2-44

TTGGAAGTCATCATGCAATTTATTTATAGTATTATTGGTATTTTATTGGTAT
TAGGAATTGTGTATGCAATTTCTTTCAATCGTAAGAGTGTTTCTCTAAGTTT
AATTGGAAAAGCTCTTATCGTTCAATTCATTATTGCGCTAATCTTAGTACGT
ATCCCACTAGGCCAACAAGTTGTTAGTGTTGTTTCAACTGGAGTTACTAAA
GTAATCAACTGTGGTCAAGCTGGTTT

MEVIMQFIYSIIGILLVLGIVYAISFNRKSVSLSLIGKALIVQFIIALILVRIPLGQQ
VVSVVSTGVTKVINCGQAG

FIG. 1CONT'D

Sequence description

A] Length:233 bp - 77 aa (partial sequence)
B] TTG start codon is preceded by a possible Shine Dalgarno sequence. Actual start codon may occur further downstream. Potential leader peptide.

ID-168

Clone 2-46

CAACCTAATAAAGCTTTAGAAAGTGATGAGATTGATATTAATGCTTTCCAG
CATTATAATTACTTAACCAATTGGAATAAAGCAAATAAGACCAATCTTGTT
TCCGTTGCTGAGACATACTTTACTTCCTTTAGATTATACTCTGGTACTAAGA
ACGGTAAAGGTAAATACCAAACAGTTTCTGAAATTCCAAATAAAGCAACT
ATTACTATCCCAAACGATGCAGTTAACGAAAGTCGCTCTCTCTACTTGTTA
CAATCAGCAGGCTTGCTAAAATTGAAAGTATCAGGTGATACATTAGCAAC
AATGTCAGATGTTGTTTCCAATCCTAAATCTTTAGATTT

QPNKALESDEIDINAFQHYNYLTNWNKANKTNLVSVAETYFTSFRLYSGTKN
GKGKYQTVSEIPNKATITIPNDAVNESRSLYLLQSAGLLKLKVSGDTLATMSD
VVSNPKSLD

Sequence description

A] Length:344 bp - 114 aa (partial sequence)
B] N- and C- termini require verification

ID-169

Clone 2-47

ATGAAATGTATAATAAATAATATAAATAAAATAAAAATGATAATTGAGAT
TTATCATAGAAGGAAAACTATTTTGAAATTAAATAAAATCATATTATCTAC

FIG. 1 CONT'D

TGCAGCTCTTACTGCTCTCTTTTTAGGATATAATAGCGTTACTGCGGATACA
TATAATAACTATCAGCCACATAGATCAAATAATATGGATTTAACTGAGGA
ATATAACTATAATAACCAGATAGAACTTCAGGAGCGTATAAAAAACCTAA
ATATACCTTTT

MKCIINNINKIKMIIEIYHRRKTILKLNKIILSTAALTALFLGYNSVTADTYNNY
QPHRSNNMDLTEEYNYNNQIELQERIKNLNIPF

Sequence description

A] Length:264 bp - 88 aa (partial sequence)
B] There is a Shine-Dalgarno sequence upstream of this sequence. Potential leader peptide sequence

ID-169

Clone 2-47

ATGAAATGTATAATAAATAATATAAATAAAATAAAAATGATAATTGAGAT
TTATCATAGAAGGAAAACTATTTTGAAATTAAATAAAATCATATTATCTAC
TGCAGCTCTTACTGCTCTCTTTTTAGGATATAATAGCGTTACTGCGGATACA
TATAATAACTATCAGCCACATAGATCAAATAATATGGATTTAACTGAGGA
ATATAACTATAATAACCAGATAGAACTTCAGGAGCGTATAAAAAACCTAA
ATATACCTTTT

MKCIINNINKIKMIIEIYHRRKTILKLNKIILSTAALTALFLGYNSVTADTYNNY
QPHRSNNMDLTEEYNYNNQIELQERIKNLNIPF

Sequence description

A] Length:264 bp - 88 aa (partial sequence)
B] There is a Shine-Dalgarno sequence upstream of this sequence. Potential leader peptide sequence

FIG. 1 CONT'D

ID-170

Clone RS-58b

TTGGGTGATTATTATGGTAAGAAATATTTTGGTGAGGCAGCTAAAAAAGA
CGTCGAACATATGGCTAAGAAAATCATTAATGTCTATAAAACACGGTTAA
AAAACAACACTTGGTTATC
AGAAAATACAAAAGCAATGGCCATTAAGAAACTTGATAACATGAGATTAA
TGATTGGCTATCCAGAAGATTATCCTGATCTTTATCGTCAGTACCAATTTG
ATAGTAAAGCAAGCTTCTTTGAAAACAATGATAACTACAGAAAATTATCG
AACAAGAAAACATTTGAAGAATTTAACCAGTCTAATCAACGTGAACATTG
GCAAATGAGTGCCAATGCTGTAAATGCTTATAATGATCCTAATACCAATTC
CATAGTCTTTCCAGCAGCGATTTTTCAATCACCACTGTACGATAAAACTAA
AACAGTTAGTCAAAATTATGGAGCTATCGGAGCAATTATTGGTCATGAAAT
TTCACACTCATTTGATATTAATGGTATGAAATATGACGAGAAAGGGAATCT
TCACGATTGGTGGACTAAAGAAGATTTAAATCATTATAAGAAATCAACAC
AAGCTATGATTGACCAATGGGATGGCCTTAAAGCAGATGGCGGTAAAGTT
GATGGTAAATTAACTTTAGCAGAAAATATTGCAGATAATGGTGGTGTTATG
GCATCTCTAGAAGCTCTTAAGACTGAAAAAATCCAAACTATAAAGAATTTT
TTGAATCATGGGCAAGTATTTGGCGTCAAAAAGCAACCAAAGAACAAAGT
AAGTCCTCAATTCAGTCAGATGTTCATGCACCATATGAATTGA >
GAGCTAACATCCCAGTACGTAATTTCCAAGAATTTTATGATGCCTTTGGTG
TTAAAAAAGGCGATTCAATGTATCTAAAACCAGAAAAACGTTTGACACTTT
GGTAA

MGDYYGKKYFGEAAKKDVEHMAKKIINVYKTRLKNNTWLSENTKAMAIKK
LDNMRLMIGYPDYPDLYRQYQFDSKASFFENNDNYRKLSNKKTFEEFNQSNQ
REHWQMSANAVNAYNDPNTNSIVFPAAIFQSPLYDKTKTVSQNYGAIGAIIGH
EISHSFDINGMKYDEKGNLHDWWTKEDLNHYKKSTQAMIDQWDGLKADGG
KVDGKLTLAENIADNGGVMASLEALKTEKIQTIKNFLNHGQVFGVKKQPKNK
VSPQFSQMFMHHMN*

Sequence description:

A] Length: 819 bp - 272 aa (full length gene)
(107 bp of additional DNA sequence (> onwards) is
also included. While not in-frame with the
described orf, it also shares strong homology
with the neutral peptidases.

FIG. 1 CONT'D

B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-89 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-89 gene sequence. ID-89 and ID-170 together show homology over their combined entire length with the neutral endopeptidases from Lactococcus and Lactobacillus. Possesses TTG (possible ATG start codon located 13 bp further downstream) start codon with no obvious signal peptide. Shine Dalgarno sequence not immediately obvious. Possibly located further downstream

ID-171

Clone 2-18/22b (Mod2)

ATGACCATGATTACGCCAAGCTTCATTAAGGTATCTCTAGATGAAACAAAT
CGTATGATGCGTATGATATCAGATTTATTAAGTTTATCGCGCATTGATAAT
GAAGTAACGCATTTAGATGTTGAAATGACGAATTTTACAGCTTTCATGACC
TCAATTTTGAATCGATTTGATCAGATTAGAAATCAAAAAACAGTCACAGG
AAAAGTTTATGAAATTGTCAGAGATTATCCTCTTAAGTCAATTTGGGTGGA
AATTGATACAGATAAGATGACTCAAGTGATTGATAACATTTTAAATAATGC
AGTCAAGTATTCACCAGATGGTGGTAAGATTACAGTTAATCTACGCACAAC
TAAAACGCAGATGATTTTATCAATATCAGACCAAGGCTTAGGTATTCCCAA
AAAAGATTTACCTCTCATTTTTGATCGTTTTTATCGTGTTGATAAGGCGAGA
AGTCGTCAACAGGGTGGGACTGGACTTGGTTTGTCAATTGCAAAAGAAAT
TGTTAAGCAGCATAAGGGATTTATTTGGGCTAAGAGTGAGTATGGTAAAG
GGTCTACTTTTACAATCGTCTTGCCTTATGATAAAGATGCTGTAACTTATGA
AGAATGGGAGGACGTTGAAGATTAA

MTMITPSFIKVSLDETNRMMRMISDLLSLSRIDNEVTHLDVEMTNFTAFMTSIL
NRFDQIRNQKTVTGKVYEIVRDYPLKSIWVEIDTDKMTQVIDNILNNAVKYSP
DGGKITVNLRTTKTQMILSISDQGLGIPKKDLPLIFDRFYRVDKARSRQQGGTG
LGLSIAKEIVKQHKGFIWAKSEYGKGSTFTIVLPYDKDAVTYEEWEDVED*

Sequence description:

A] Length: 613 bp - 212 aa (full-length gene possibly)
B] Possible Shine Dalgarno sequence present upstream of a ATG start codon. May not have yet determined the N- portion of this gene. No obvious signal peptide.

FIG. 1CONT'D

ID-172

Clone 2-54balternate (107b)

TTGAAAAAAATTATTACTTCTATTCTATTACTTAGTTGCATTTTTTTTATGC
CAACCATCTCTGCTGAATCTTTTAATGCTTCCGCTAAACATGCCTTAGCAGT
TGATTTAGATTCAGGAAAAATCTTGTATGAAAAAGATGCTAACAAACCCG
CTGCTATTGCTTCCTTGACTAAAATAATGACCGTTTATATGGTCTATAAAG
AAATTGATAACGGTAACCTCAAGTGGAATACCAAAGTAAATATATCTGAC
TACCCTTATCAACTAACACGCGAATCTGATGCTAGTAATGTTCCTTTAGAA
AAAAGGCGCTATACTGTTAAACAACTCGTGGACGCTGCCATGATTTCTAGT
GCTAACAGTGCAGCCATTGCTTTAGCTGAACATATTTCAGGAACTGAAAGT
AAATTTGTTGATAAAATGACTGCTCAATTGGAAAAGTGGGGAATTCATGAT
AGCCACCTAGTCAATGCTTCTGGCTTAAATAATAGTATGTTAGGCAATCAC
ATTTATCCAAAATCGTCACAAAACGACGAAAATAAAATGAGTGCACGTGA
TATTGCTATTGCTGCCTACCATTTGGTCAACGAATATCCTTCCATTCTTAAG
ATTACTAGTAAGTCCGTTGCTAAATTTGATAAAGATATTATGCATTCTTAT
AACTACATGCTACCAGATATGCCTGTCTTTAGACCAGGTATTACAGGTTTG
AAAACTGGGACAACGGAATTAGCTGGCCAATCTTTTATTGCTACATCTACT
GAAAGTGGAATGAGACTACTCACTGTTATTATGCATGCTGATAAGGCCGAT
AAAGACAAATATGCTCGCTTTACAGCAACTAACTCTCTCTTGAACTATATC
ACAAACACCTACGAACCTAACCTTGTATTAGCTAAAGGAGCTGCATATAA
AGGTAAAGAAGCAAGTGTGAGAGACGGAAAAGAACAATCGGTCATCGCT
GTTGCTAAAAACGATTTGAAAGTAGTACAGAAGAAAAATATCACTAAACA
AAATCAGTTAAAAATTAACTTTAAAAAAGAGCTTACTGCTCCTATTACAAA
AAAAGAGAACCTAGGGAAAGCTTATTACGTTGACCTTAATAAGGTTGGAA
AAGGCTATCTCATAAAGGAACCTAGCGTTCATTTAGTGGCAAAAGATAGT
ATTGAGCGCAGTTTCTTCCTCAAAGTGTGGTGGAATCATTTTGTGCGCTAC
GTTAACGAAAAACTTTAA

MKKIITSILLLSCIFFMPTISAESFNASAKHALAVDLDSGKILYEKDANKPAAIA
SLTKIMTVYMVYKEIDNGNLKWNTKVNISDYPYQLTRESDASNVPLEKRRYT
VKQLVDAAMISSANSAAIALAEHISGTESKFVDKMTAQLEKWGIHDSHLVNA
SGLNNSMLGNHIYPKSSQNDENKMSARDIAIAAYHLVNEYPSILKITSKSVAKF
DKDIMHSYNYMLPDMPVFRPGITGLKTGTTELAGQSFIATSTESGMRLLTVIM
HADKADKDKYARFTATNSLLNYITNTYEPNLVLAKGAAYKGKEASVRDGKE
QSVIAVAKNDLKVVQKKNITKQNQLKINFKKELTAPITKKENLGKAYYVDLN
KVGKGYLIKEPSVHLVAKDSIERSFFLKVWWNHFVRYVNEKL*

FIG. 1CONT'D

Sequence description:

A] Length: 1236 bp - 412 aa (full-length gene sequence possibly)
B] A possible Shine-Dalgarno sequence precedes the putative 'TTG' start codon. (needs further cloning and sequencing to verify N-terminus)

ID-173

Clone 3-60b

ATGACGCTTCGAGAATTAACAATAGAAGAATTTAAAGAACATTCAGGAAA
TTATGATTCACAATCATTTTTACAAACACCTGAGATGGCTAAACTTTTAGA
AAAACGCGGCTATGATGTTAGGTATTTGGGATATCAAGTAGAAAATAAAC
TAGAGATAATCAGTTTATCTTATATTATGCCAGTCACTGGTGGTTTTCAAAT
GAAAATTGATTCAGGACCAGTTCATTCAAATTCTAAGTATCTAAAACAATT
TTATAAAGCATTGCAAGGCTATGCCAAATCCAACGGTGTTCTAGAATTAAT
AGTTGAGCCTTTTGATGATTACCAATTATTCACTAGTTCGGGAGTTCCTAGT
AATCAGGGAAATGATAATCTGATTGAAGATTTTACCAGTTCAGGTTATCAC
CATGATGGTTTAACAACTGGTTTTACTGGTAAATATTTATCTTGGCACTATG
TTAAAAATTTAGAAGGTGTCACTTCTGAAACGTTACTATCTTCATTCTCTAA
GACAGGACGAGCTTTGGTTAAGAAAGCAATGTCTTTTGGAATCAAGGTTC
GCGTTCTTAAACGTGATGAGCTACATTTATTTAAAGAGATAACAACTTCTA
CGTCAAATAGACGTGATTATATGGATAAGTCCTTAGATTATTATCAAGATT
TTTACGATAGCTTTGAAGGCAAGGCTGAATTTGTGATTGCCACTTTAAATT
TTAGAGAATACGACCATAACTTGCAAATAAAAGCTGAAGCATTGGAAAAT
AAGCTT

MTLRELTIEEFKEHSGNYDSQSFLQTPEMAKLLEKRGYDVRYLGYQVENKLEI
ISLSYIMPVTGGFQMKIDSGPVHSNSKYLKQFYKALQGYAKSNGVLELIVEPF
DDYQLFTSSGVPSNQGNDNLIEDFTSSGYHHDGLTTGFTGKYLSWHYVKNLE
GVTSETLLSSFSKTGRALVKKAMSFGIKVRVLKRDELHLFKEITTSTSNRRDY
MDKSLDYYQDFYDSFEGKAEFVIATLNFREYDHNLQIKAEALENKL

Sequence description
A) Length: 771 bp - 257 aa (partial gene sequence)
B) This gene sequence was not identified using the LEEP system. It was identified immediately downstream of the ID-65 gene which was identified by

FIG. 1 CONT'D

LEEP, during cloning and sequence analysis of the full-length ID-65 gene sequence. Sequence Characteristics:
No obvious leader peptide sequence
Orf is preceded by a potential Shine-
Dalgarno sequence.

ID-174

Clone 2-17b (ID-80b)

TTGTCATTAAGTTTGGTTGCAGTGTTAAATCTTATCCCTCCTAAAATCATGG
GATCAGTTATTGATGCTATTACAACTGGAAAATTAACAAGACCACAATTAC
TATGGAATTTATTAGGTTTGGTTTTGTCAGCTTTAGCTATGTATGGGCTGCG
TTATATTTGGCGTATGTATATTTTAGGGACTTCTTACAAATTAGGCCAAGTT
GTCAGATACCGTTTATTTGAACATTTTACAAAAATGTCTCCTTCTTTTTATC
AGAAATATCGTACAGGTGATTTAATGGCGCACGCGACCAACGACATCAAT
TCTCTAACACGTCTTGCAGGAGGAGGAGTTATGTCAGCAGTGGATGCCTCT
ATCACAGCATTAGTAACGCTTATCACCATGTTCTTTACTATTTCGTGGCAA
ATGACATTAATTGCGGTTATCCCTTTGCCCTTAATGGCCTTAGCACTAGTA
AATTGGGGCGAAAAACCCATGAAACCTTCAAAGAATCTCAGGCAGCCCTT
TTCAGAATTAAATAATAAAGTG

MSLSLVAVLNLIPPKIMGSVIDAITTGKLTRPQLLWNLLGLVLSALAMYGLRYI
WRMYILGTSYKLGQVVRYRLFEHFTKMSPSFYQKYRTGDLMAHATNDINSLT
RLAGGGVMSAVDASITALVTLITMFFTISWQMTLIAVIPLPLMALALVNWGEK
PMKPSKNLRQPFSELNNKV

Sequence description
A) Length: 534 bp - 178 aa (partial gene
sequence)
B) This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-80 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-80 gene sequence. Sequence Characteristics:
No obvious leader peptide sequence
Orf is preceded by a potential Shine-
Dalgarno sequence.

FIG. 1CONT'D

ID-175

Clone 2-11Ab (ID-103b)

ATGCATATTGAGACTGTTATTGATTTCAAAGAATTAGGAAAAAGATATCGT
TTTAAAAATCCTACAAAAGAATTAATAGCTGATACTTTAGAACAAGTCTTA
GAAGTGATAAAAGAAGTTGATTATTATCAATCTCAAAATTATTATGTTGTT
GGTTATTTATCTTATGAAGCATCTGCTGCTTTTGATTCACATTTTAAAGTTT
CTCAACAGAAGTTGGCTGGAGAACATCTAGCTTATTTTACAGTACATAAAG
ATTGTGAGAACGAAGCTTTTCCTTTAAGTTATGAAAATGTTAGATTAGCAG
ATAATTGGACTGCTAATGTTTCTGAGCAAGAATATCAAGAGGCAATTGCTA
ATATTAAAGGACAAATTAGACAAGGAAATACTTATCAAGTAAATTATACA
CTAGAGCTTAGCCAACAATTATGCTCGGATCC

MHIETVIDFKELGKRYRFKNPTKELIADTLEQVLEVIKEVDYYQSQNYYVVGY
LSYEASAAFDSHFKVSQQKLAGEHLAYFTVHKDCENEAFPLSYENVRLADNW
TANVSEQEYQEAIANIKGQIRQGNTYQVNYTLELSQQLCSD

Sequence description:

A] Length: 440 bp - 146 aa (partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-103 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-103 gene sequence. Shine Dalgarno sequence present upstream of
ATG start codon, No apparent leader peptide sequence

ID-176

Clone 2-18/22b(b) (ID-104b)

GTGAATAATATGTTTTATCTCAAAATAGCCTGGCATAATTTAAAACATTCT
ATAGACCAGTACATACCATTCCTCTTAGCCAGTTTATTACTTTATTCATTGA
CTTGTTCTACGCTACTAATCTTAATGAGTGCTGTTGGAAGAGATATGGGGA
CAGCGGCAACGGTTCTTTTTCTTGGAGTGATTGTTTTGTCAATCTTTGCGGT
AGTCATGGAACATTATAGCTACAATATCTTGATGAAACAGCGTAGTAGTG

FIG. 1CONT'D

AATTTGGACTGTATAACATTTTGGGGATGAATAAACGTCAAGTTGCGCGTG
TAGCTAGTCTAGAGCTGTTTATTATTTATATATTTCTTATTTCTATAGGAAG
TCTGTTTAGTGCTTTTTTTGCTAAATTTATTTATTTAATTTTTGTCAACATTA
TTAACTATCATGCACTAAATCTTAGTTTAAGTTTATGGCCATTTATTATTTG
TATCGTTATATTTACAGGTATTTTTCTGACTTTAGAAGTTCCAGTTATTCGA
CATGTTCATTTATCATCCCCATTAAGTCTTTTTAGAAAGAAACAACAGGGA
GAAAAAGAACCAAAAGGTAATCTTATACTTGCAATTTTAGCGTTAGTAGCT
ATCGCCATCGCTTATACAATGGCTCTTACTTCAGGTAAAGCACCTGCATTA
GCTGTTATCTATCGTTTCTTCTTTGCAGTACTTTTAGTAATTGCTGGTACTT
ATCTTTTTTATATTAGTTTTATGACATGGTACTTAAAAAGGTTGCGTCAAAA
CAAGCATTATTATTATAAATCTGAGCATTTTGTATCAACTTCGCAAATGAT
TTTTCGAATGAAGCAAAATGCAGTAGGGTTAGCAAGTATCACTTTATTAGC
TGTTATGGCTCTAGTTACTATTGCTACAACAGTCTCACTCTATTCAAATACA
CAAAATGTTGTTACCGGACTATTTCCAAAATCAGTAAGTTTATCAATAGAT
AATTCAAAAGGTGACGCGAAAAATATATTTGAAGAAAAGATTTTGAAGAA
ACTAGGTAAGTCATCTAAGGAAGCTATCACTTATAATCAGACAATGATTTC
GATGCCAGTTAGTCAATCAAGTGACTTAATATCACATCTA

MNNMFYLKIAWHNLKHSIDQYIPFLLASLLLYSLTCSTLLILMSAVGRDMGTA
ATVLFLGVIVLSIFAVVMEHYSYNILMKQRSSEFGLYNILGMNKRQVARVASL
ELFIIYIFLISIGSLFSAFFAKFIYLIFVNIINYHALNLSLSLWPFIICIVIFTGIFLTLE
VPVIRHVHLSSPLSLFRKKQQGEKEPKGNLILAILALVAIAIAYTMALTSGKAP
ALAVIYRFFFAVLLVIAGTYLFYISFMTWYLKRLRQNKHYYYKSEHFVSTSQM
IFRMKQNAVGLASITLLAVMALVTIATTVSLYSNTQNVVTGLFPKSVSLSIDNS
KGDAKNIFEEKILKKLGKSSKEAITYNQTMISMPVSQSSDLISHL

Sequence description:

A] Length: 1119 bp - 373 aa (partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-104 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-104 gene sequence. Possible Shine Dalgarno sequence present
upstream of a GTG start codon. Possesses a potential
leader peptide sequence

ID-177

FIG. 1CONT'D

Clone 2-5b (ID-112b)

ATGGTTGAGCCAATTATTTCAATACAAGGACTTCATAAAAGTTTTGGGAAA
AATGAGGTTTTAAAAGGCATTGACTTGGATATTCATCAAGGAGAAGTGGT
GGTTATTATTGGCCCTTCTGGCTCTGGTAAGTCAACATTTTTAAGAACAAT
GAATCTCTTGGAAGTACCAACAAAGGGAACAGTGACTTTTGAAGGGATTG
ATATAACAGACAAAAAGAATGATATTTTTAAAATGCGCGAAAAAATGGGC
ATGGTTTTTCAACAGTTCAATCTATTTCCCAATATGACTGTACTAGAAAAT
ATTACTTTATCACCTATTAAGACAAAGGGACTTTCTAAGCTTGATGCTCAG
ACAAAAGCATACGAGCTACTTGAAAAAGTTGGACTCAAAGAGAAGGCTAA
TGCTTATCCAGCAAGCTTATCTGGAGGACAACAACAACGGATTGCTATTGC
AAGAGGTCTTGCAATGAATCCTGATGTCCTTCTTTTTGATGAACCTACTTCA
GCTCTTGATCCTGAAATGGTAGGTGAAGTCTTGACTGTTATGCAAGATTTA
GCTAAATCTGGTATGACGATGGTTATTGTCACTCATGAAATGGGTTTTGCA
CGTGAAGTAGCGGATCGTGTCATTtTTATGGATGCAGGGATTATTGTTGAG
CAAGGGACCCCTAAGAAAGTATTTGAGCAGACAAAAGAAATCCGCACAAG
AGACTTCTTAAGTAAAGTATTATAA

MVEPIISIQGLHKSFGKNEVLKGIDLDIHQGEVVVIIGPSGSGKSTFLRTMNLLE
VPTKGTVTFEGIDITDKKNDIFKMREKMGMVFQQFNLFPNMTVLENITLSPIKT
KGLSKLDAQTKAYELLEKVGLKEKANAYPASLSGGQQQRIAIARGLAMNPDV
LLFDEPTSALDPEMVGEVLTVMQDLAKSGMTMVIVTHEMGFAREVADRVIF
MDAGIIVEQGTPKKVFEQTKEIRTRDFLSKVL*

Sequence description:

A] Length: 735 bp - 244 aa (full length gene)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence precedes the 'ATG'
start codon. No obvious leader peptide

ID-178

Clone 2-5c (ID-112c)

FIG. 1CONT'D

ATGTCTCAsTATCAAGAGTGGTTAGAAAACGACTCACTCGGTAAAGATATT
AAGTCAGATTTAGAAGCTATTAAAGGAGATGAATCTGAAATTCAGGATCG
TTTTTACAAAACATTAGAATTTGGAACGGCGGGATTGAGAGGTAAACTTG
GAGCAGGAACCAATCGTATGAATACTTATATGGTGGGGAAAGCAGCACAA
GCATTAGCTAATCGATTATTGATCATGGCCCTGAAGCTATTGCACGTGGAA
TTGCAGTTAGTTATGATGTCCCGTTATCAATCTAAGGAATTTGCAGAATTA
ACTTGGTCCATTATGGCAGCAAATGGTATTAAAGCCTTATATTTA

MSHMNYKEIYQEWLENDSLGKDIKSDLEAIKGDESEIQDRFYKTLEFGTAGLR
GKLGAGTNRMNTYMVGKAAQALANRLLIMALKLLHVELQLVMMSRYQSKE
FAELTWSIMAANGIKALYL

Sequence description:

A] Length: 366 bp - 122 aa (partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence preceded the 'ATG'
start codon. No obvious potential leader peptide sequence.

ID-179

Clone 2-5d (ID-112d)

ATGCAACCTGTAAAAGTCGATGAACCTTCTGTTGAAGAAACCATTACTATT
TTGAAAGGTATCCAAAAAAAATACGAAGATTATCATCACGTAAAATATAA
TAATGATGCCATAGAAGCAGCTGCAGTACTATCTAATCGTTATATCCAAGA
CCGCTTTTTACCTGATAAAGCAATAGACTTATTAGATGAAGCTGGTTCTAA
AATGAACCTAACACTAAATTTTGTTGATCCAAAAGAAATTGATCAACGTCT
CATTGAAGCAGAAAATTTAAAAGCGCAAGCGACTCGTGAAGAAGATTACG
AACGTGCAGCTTACTTCCGTGACCAGATTGCAAAATATAAAGAAATGCAG
CAACAAAAGGTCGACGATCAAGATACACCTATTATTACCGAAAAAACAAT
TGAGCACATCATTGAAGAAAAAACGAATATCCCTGTTGGTGATTTAAAAG
AAAAAGAACAATCTCAATTAATTAATCTCGCAGATGACTTGAAACAGCAT
GTGATCGGCCAGGATGACGCTGTCATTAAGATTGCAAAAGCTATTCGTCGT
AATCGAGTTGGTCTTGGTAGCCCAAACCGTCCTATTGGTTCCTTTTTATTTG
TAGGACCAACCGGTGTTGGTAAAACTGAACTTTCTAAACAACTAGCAATTG
AGCTCTTTGGTTCAGCTGATAGTATGATTCGTTTTGATATGTCAGAGTACAT
GGAAAAGCATGCTGTTGCTAAATTAGTCGGAGCGCCTCCAGGATACGTGG
GATACGAGGAAGCTGGACAACTAACTGAAAAGGTTCGTCGAAATCCTTAC
TCGCTCATCCTTCTAGATGAAATTGAAAAAGCTCATCCCGATGTCATGCAT

FIG. 1CONT'D

ATGTTCTTGCAGGTCCTTGATGACGGTCGATTAACAGATGGACAAGGAAG
AACTGTTAGTTTTAAAGATACCATTATCATCATGACCTCAAATGCTGGTTC
TGGTAAAACTGAAGCAAGTGTTGGCTTTGGTGCCTCACGAGAAGGTAGGA
CGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCAT
GCAAGC

MQPVKVDEPSVEETITILKGIQKKYEDYHHVKYNNDAIEAAAVLSNRYIQDRF
LPDKAIDLLDEAGSKMNLTLNFVDPKEIDQRLIEAENLKAQATREEDYERAAY
FRDQIAKYKEMQQQKVDDQDTPIITEKTIEHIIEEKTNIPVGDLKEKEQSQLINL
ADDLKQHVIGQDDAVIKIAKAIRRNRVGLGSPNRPIGSFLFVGPTGVGKTELSK
QLAIELFGSADSMIRFDMSEYMEKHAVAKLVGAPPGYVGYEEAGQLTEKVRR
NPYSLILLDEIEKAHPDVMHMFLQVLDDGRLTDGQGRTVSFKDTIIIMTSNAGS
GKTEASVGFGASREGRTNSSSVPGDPLESTCRHAS

Sequence description:

A] Length: 1070 bp ÿ 356 aa (Partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-112 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-112 gene sequence. Shine-Dalgarno sequence preceded the 'ATG'
start codon. No obvious potential leader peptide
sequence.

ID-180

Clone 2-7b (ID-113b)

ATGAGAGGGAAGGTTATTTACGGCACAACCCTTATAGGTCTTTTTCTATTC
TTATTTTTCTATTTTTGGATTCCTAAGCATCACATCGAGAGAATACATCATC
ATCGTATAAAGCAGGTAGATGCGAAGAGTGATTTAACAGGATTTAAAACC
CATTTGCCCATTATCAGCATTGATACAAAGCAACAAGTTATTCCTCTTGTT
ACAAAAGAAGGCGGAAAATATGTCAAAGCTAGGGATAATATTAATGTTGA
TATCGAATTACGGGATTCTCCAAGTAGATCACATCATTTATCAGAAAAGCC
GAGAATTAGGACAAAAGGGTTAATATCATATAGAGGAAATTCCTCTCGTT
ACTTTGATAAGAAGTCATTGAAAGTTAAGTTTGTTACTAATAAGTTAAAGG
AAAAGAAGCATCGATTAGCAGGAATGCCTAAAGAATCGGAGTGGGTATTG
CATGGTCCCTTTCTAGACAGAACATTATTAAGAAATTATCTGAGTTATAAT

FIG. 1 CONT'D

ATTGCTGGTGAGATTATGCCTATGCCCCAAACGTTCGCTACTGTGAGTTAT
TTGTCAATGGTGAGTATCAGGGAG

MRGKVIYGTTLIGLFLFLFFYFWIPKHHIERIHHHRIKQVDAKSDLTGFKTHLPII
SIDTKQQVIPLVTKEGGKYVKARDNINVDIELRDSPSRSHHLSEKPRIRTKGLIS
YRGNSSRYFDKKSLKVKFVTNKLKEKKHRLAGMPKESEWVLHGPFLDRTLLR
NYLSYNIAGEIMPMPQTFATVSYLSMVSIRE

Sequence description:

A] Length: 582 bp - 194 aa (Partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-113 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-113 gene sequence. ATG start codon is preceded by a Shine-
Dalgarno sequence-Possesses a potential leader peptide
sequence. C-terminus to be determined.

ID-181

Clone 2-17b (ID-117b)

CTTCACATTTTATTGATCACTATCTGACAAATGTTAATCAAACAGCAGTTCT
TATTTTAGTGGGATATTATTCAATGTATGTCTTGCAGACCTTAATTCAATAT
TTTGGGAATCTCTTTTTTGCGCGTGTTTCTTATAGTATTGTTAGAGATATTC
GTAGAGATGCTTTTGCTAATATGGAAAGGCTAGGCATGTCTTATTTTGATA
GGACACCGGCAGGATCTATTGTGTCACGTATTACTAATGATACTGAAGCAA
TATCTGATATGTTTTCGGGTATTTTATCAAGTTTTATCTCGGCGATATTTAT
TTTTACAGTTACTCTGTACACTATGTTGATGCTAGACATTAAACTAACAGG
ACTCGTCGCTCTTTTGTTACCTGTTATCTTTATATTAGTGAATGTCTATCGG
AAAAAATCAGTCACTGTCATTGCTAAAACGAGAAGTTTACTTAGTGATATC
AACAGTAAATTATCAGAAAGTATTGAAGGAATTC

SHFIDHYLTNVNQTAVLILVGYYSMYVLQTLIQYFGNLFFARVSYSIVRDIRRD
AFANMERLGMSYFDRTPAGSIVSRITNDTEAISDMFSGILSSFISAIFIFTVTLYT
MLMLDIKLTGLVALLLPVIFILVNVYRKKSVTVIAKTRSLLSDINSKLSESIEGI

FIG. 1CONT'D

Sequence description:

A] Length: 498 bp - 165 aa (Partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-117 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-117 gene sequence. N- and C-termini have yet to be determined

ID-182

Clone 3-8b (ID-120b)

ATGTACCATATTGAATTAAAAAAGGAAGCTTTACTACCAAGAGAACGCCT
AGTTGATTTAGGCGCAGATAGATTGAGTAATCAGGAGTTATTAGCCATTCT
CTTACGTACAGGTATTAAAGAAAAACCTGTTCTTGAAATTTCAACGCAAAT
TTTAGAAAACATAAGCAGTTTAGCAGATTTTGGTCAATTATCCTTACAGGA
GTTGCAATCCATTAAAGGAATCGGTCAGGTTAAATCCGTCGAAATAAAAG
CTATGCTAGAACTAGCAAAACGGATTCACAAAGCTGAATATGATCGTAAA
GAGCAAATTTTAAGTAGTGAACAATTAGCGAGGAAAATGATGCTCGAATT
AGGGGATAAAAAACAAGAACATTTAGTAGCTATTTATATGGATACACAAA
ATCGTATTATCGAACAGAGAACTATTTTTATTGGTACTGTACGTCGTTCAG
TAGCAGAGCCAAGAGAAATTCTACATTATGCTTGTAAAAACATGGCAACT
TCTTTGATTATTATACATAATCATCCCTCAGGTTCTCCAAATCCCAGTGAAA
GTGATTTAAGTTTCACTAAAAAAATAAAACGATCATGTGATCATCTGGGAA
TTGTCTGCCTAGATCACATCATCGTTGGAAAAAATAAATATTATAGTTTTC
GAGAAGAAGCAGATATTTTATAA

MYHIELKKEALLPRERLVDLGADRLSNQELLAILLRTGIKEKPVLEISTQILENI
SSLADFGQLSLQELQSIKGIGQVKSVEIKAMLELAKRIHKAEYDRKEQILSSEQ
LARKMMLELGDKKQEHLVAIYMDTQNRIIEQRTIFIGTVRRSVAEPREILHYAC
KNMATSLIIIHNHPSGSPNPSESDLSFTKKIKRSCDHLGIVCLDHIIVGKNKYYSF
REEADIL*

Sequence description:

A] Length: 681 bp - 227 aa (full-length gene)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-120 gene which was identified by LEEP,

FIG. 1CONT'D

during cloning and sequence analysis of the full-length ID-120 gene sequence. ATG start codon is preceded by an typical Shine-Dalgarno sequence. No obvious leader peptide sequence

ID-183

Clone 3-11b (ID-121b)

TGGTTAAAAGTAGTGATAGCTTGTATTCCATCTATTTTAATTGCTTTACCAT
TTGATAATTGGTTTGAAGCTCATTTTAATTTCATGATTCCGATTGCAATAGC
CCTAATCTTTTATGGTTTTGTCTTCATATGGGTTGAAAAACGTAATGCACAC
CTCAAACCACAGGTAACCGAATTGGCAAGTATGTCTTACAAGACAGCTTTC
TTGATTGGATGTTTCCAGGTTCTCAGTATTGTTCCGGGAACCAGTCGTTCTG
GAGCTACTATTTTAGGAGCAATTATTATTGGAACTAGTCGTTCGGTCGCTG
CTGACTTTACTTTCTTCCTTGCCATCCCAACTATGTTTGGTTATAGTGGACT
TAAGGCGGTTAAATATTTTTTAGATGGTAACGTCTTGAGTTTAGACCAATC
TTTAATACTTTTAGTAGCAAGTCTGACAGCTTTCGTAGTTAGTTTATATGTT
ATTCGTTTCTTGACAGACTATGTCAAACGACACGATTTCACCATCTTTGGT
AAGTATCGTATAGTCTTAGGAAGTTTACTCATCCTCTACTGGTTAGTTGTTC
ATTTATTCTAA

WLKVVIACIPSILIALPFDNWFEAHFNFMIPIAIALIFYGFVFIWVEKRNAHLKP
QVTELASMSYKTAFLIGCFQVLSIVPGTSRSGATILGAIIIGTSRSVAADFTFFLA
IPTMFGYSGLKAVKYFLDGNVLSLDQSLILLVASLTAFVVSLYVIRFLTDYVKR
HDFTIFGKYRIVLGSLLILYWLVVHLF*

Sequence description:

A] Length: 579 bp - 193 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-68 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-68 gene sequence described in WO 00/06736. N-terminus has yet to be determined.

ID-184

Clone 3-11c (ID-121c)

FIG. 1CONT'D

ATGGAAATGAAACAAATCAGTGAAACAACACTGAAAATTACAATTAGTAT
GGAAGATTTAGAAGATCGTGGTATGGAGCTGAAAGATTTCCTAATCCCTCA
GGAGAAGACTGAGGAATTTTTCTATTCTGTCATGGATGAATTAGACTTGCC
AGAAAACTTTAAAAATAGTGGTATGTTAAGTTTTCGAGTAACACCTAAAA
AAGATCGCATTGATGTTTTTGTTACAAAGTCTGAATTAAGTAAAGATTTAA
ATTTAGAAGAATTAGCAGATTTGGGTGACATTTCAAAAATGTCTCCAGAAG
ACTTTTTTAAAACCTTGGAACAATCGATGTTGGAAAAAGGGGATACGGAT
GCCCATGCCAAATTAGCAGAAATTGAAAATATGATGGATAAAGCAACTCA
AGAAGTAGTTGAGGAAAATGTTTCTGAAGAACAACCTGAAAAGGAAGTAG
AAACGATTGGATATGTTCACTATGTCTTTGATTTTGATAATATTGAAGCTGT
AGTTCGATTTTCACAAACGATTGATTTTCCAATAGAAGCTT

MEMKQISETTLKITISMEDLEDRGMELKDFLIPQEKTEEFFYSVMDELDLPENF
KNSGMLSFRVTPKKDRIDVFVTKSELSKDLNLEELADLGDISKMSPEDFFKTLE
QSMLEKGDTDAHAKLAEIENMMDKATQEVVEENVSEEQPEKEVETIGYVHY
VFDFDNIEAVVRFSQTIDFPIEA

Sequence description:

A] Length: 547 bp - 182 aa (Partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-68 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-68 gene sequence. ATG start codon is preceded by an typical
Shine-Dalgarno sequence. No obvious potential
leader peptide
sequence

ID-185

Clone 3-16b (ID-122b)

GGAAACCAACGGCCAGTACAATCGTCAAGGGTAGATTATCCTAAACGTAG
TCGTGCCAAGATTGTAGAAGTTTATTTTAGACAAGCTTCTACTACTGATTA
TTCTGGTGTTTACAAAGGTTACTATATTGACTTTGAAGCCAAAGAAACCCG
GCAGAAAACTGCTATGCCTATGAAAAATTTTCATGCTCACCAAATAGAGC
ACATGGCAAATGTATTACAGCAAAAAGGGATTTGCTTTGTCTTGCTTCATT

FIG. 1 CONT'D

TTTCCACACTTAAGGAAACCTATCTACTCCCTGCTAATGAGTTAATTTCATT
TTATCAGATTGATAAAGGCAATAAATCAATGCCTATTGATTATATCAGAAA
AAATGGATTTTTCGTAAAGGAGAGTGCCTTTCCTCAAGTCCCTTACTTAGA
TATTATTGAAGAAAAATTATTAGGCGGTGATTACAATTAA

GNQRPVQSSRVDYPKRSRAKIVEVYFRQASTTDYSGVYKGYYIDFEAKETRQ
KTAMPMKNFHAHQIEHMANVLQQKGICFVLLHFSTLKETYLLPANELISFYQI
DKGNKSMPIDYIRKNGFFVKESAFPQVPYLDIIEEKLLGGDYN*

Sequence description:

A] Length: 447 bp - 149 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-122 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-122 gene sequence. N-terminus has yet to be determined

ID-186

Clone 3-17b (ID-123b)

GGATCCTAAAAACGCTAAGGTTTATCAAAAAAATGCTGATCAATTTAGTG
ACAAGGCAATGGCTATTGCAGAGAAGTATAAGCCAAAATTTAAAGCTGCA
AAGTCTAAATACTTTGTGACTTCACATACAGCATTCTCATACTTAGCTAAG
CGATACGGATTGACTCAGTTAGGTATTGCAGGTGTCTCAACCGAGCAAGA
ACCTAGTGCTAAAAAATTAGCCGAAATTCAGGAGTTTGTGAAAACATATA
AGGTTAAGACTATTTTTGTTGAAGAAGGAGTCTCACCTAAATTAGCTCAAG
CAGTAGCTTCAGCTACTCGAGTTAAAATTGCAAGTTTAAGTCCTTTAGAAG
CAGTTCCCAAAAACAATAAAGATTACTTAGAAAATTTGGAAACTAATCTTA
AGGTACTTGTCAAATCGTTAAATCAATAG

DPKNAKVYQKNADQFSDKAMAIAEKYKPKFKAAKSKYFVTSHTAFSYLAKR
YGLTQLGIAGVSTEQEPSAKKLAEIQEFVKTYKVKTIFVEEGVSPKLAQAVAS
ATRVKIASLSPLEAVPKNNKDYLENLETNLKVLVKSLNQ*

Sequence description:

FIG. 1 CONT'D

A] Length: 433 bp - 144 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-123 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-123 gene sequence. N-terminus has yet to be determined

ID-187

Clone 3-46/47 (ID-130b)

ATGAAAAAAGTCATCGATTTAAAAAAACTACAAAAAGCATACGCCTCAGA
AACTGTTTTAAATAATATTAATTTGGAGGTGTTTAAAGGAGAAATAATTGG
ATTAATAGGACCCTCTGGAGCAGGGAAATCTACCTTGATTAAAACTATGCT
TGGCATGGAAAAAGCAGATAAGGGAACAGCTCTTGTTCTTGATACTCAAA
TGCCAGATCGTAATATTTTAAATCAAATTGGCTATATGGCTCAATCTGATG
CCTTACACGAGTCTTTAACTGGCTTAGAAAATTTATTATTCTTTGGAAAAA
TGAAAGGTATTCAAAAAACTGAATTAAAACAGCAGATAACTCATATTTCT
AAAGTAGTAGATCTAGAAAACCAACTTGATAAATTTGTCTCAGGTTACTCA
GAAGGTATGAAAAGACGGCTTTCTCTAGCCATCGCCCTACTTGGAAACCCC
ACAGTTTTAATCCTAGATGAACCTACCGTTGGAATTGATCCATCCTTGAGG
AGAAAAATCTGGCAAGAGCTAATTAATATTAAGGATGAAGGACGTTCTAT
CTTTATTACAACCCACGTTATGGATGAAGCAGAATTAACAAGTAAGGTTGC
ACTACTATTACGTGGAAACATTATTGCCTTTGATACTCCATTACATTTAAA
AAAACAATTTAATGTGAGTACTATTGAGGAAGTTTTCTTAAAAGCTGAAGG
AGAATAA

MKKVIDLKKLQKAYASETVLNNINLEVFKGEIIGLIGPSGAGKSTLIKTMLGME
KADKGTALVLDTQMPDRNILNQIGYMAQSDALHESLTGLENLLFFGKMKGIQ
KTELKQQITHISKVVDLENQLDKFVSGYSEGMKRRLSLAIALLGNPTVLILDEP
TVGIDPSLRRKIWQELINIKDEGRSIFITTHVMDEAELTSKVALLLRGNIIAFDTP
LHLKKQFNVSTIEEVFLKAEGE*

Sequence description:

A] Length: 717 bp - 239 aa (Possible full-length sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-130 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-130 gene sequence. ATG start codon is preceded by a possible

FIG. 1 CONT'D

Shine-Dalgarno. No obvious potential leader peptide sequence

ID-188

Clone 3-83b (ID-144b)

ATGGTACAAATGATACATGATATGATTAAAACAATTGAGCATTTTGCTGAG
ACACAAGCTGATTTTCCAGTGTATGATATTTTAGGGGAAGTCCATACTTAT
GGACAACTTAAAGTAGACTCTGACTCTCTAGCTGCTCATATTGATAGCCTA
GGCCTTGTTGAAAAATCACCTGTCTTAGTATTCGGTGGTCAAGAATATGAA
ATGTTGGCGACATTTGTTGCTTTAACAAAGTCAGGGCATGCTTATATACCG
GTTGACCAACACTCTGCTTTGGATAGAATACAGGCTATTATGACAGTTGCT
CAACCAAGCCTTATCATTTCAATTGGTGAATTTCCTCTTGAAGTTGATAAT
GTCCCAATCCTAGACGTTTCTCAAGTTTCAGCTATTTTTGAAGAAAAGACT
CCTTATGAGGTAACACATTCTGTTAAAGGTGATGATAATTACTATATTATT
TTCACTTCAGGGACTACTGGTTTACCAAAAGGTGTGCAAATTTCACATGAC
AATTTATTGAGCTTTACAAATTGGATGATTTCTGATGATGAGTTTTCAGTTC
CTGAAAGACCGCAAATGTTGGCTCAACCC

MVQMIHDMIKTIEHFAETQADFPVYDILGEVHTYGQLKVDSDSLAAHIDSLGL
VEKSPVLVFGGQEYEMLATFVALTKSGHAYIPVDQHSALDRIQAIMTVAQPSL
IISIGEFPLEVDNVPILDVSQVSAIFEEKTPYEVTHSVKGDDNYYIIFTSGTTGLP
KGVQISHDNLLSFTNWMISDDEFSVPERPQMLAQP

Sequence description:

A] Length: 592 bp - 197 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-144 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-144 gene sequence. Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. No obvious leader peptide sequence
This orf is not in frame with nuc

ID-189

FIG. 1 CONT'D

Clone 3-86b (ID-145b)

ATGGAAAATCATCGTTATGAAGATGAAGGTAAATTCCAGCGTAAGATGAC
CAGTCGTCATCTCTTTATGTTATCGCTAGGTGGTGTTATCGGGACTGGGCTT
TTCTTGAGTTCAGGTTATACCATTGCACAGGCTGGTCCGCTTGGAGCTGTG
CTGTCTTATTTGATTGGTGCCGTTGTGGTTTATTTGGTCATGCTATCACTTG
GGGAATTGGCGGTTGCCATGCCGGTGACGGGGTCATTCCACACTTATGCCA
CTAAGTTTATCAGTCCTGGAACAGGTTTTACTGTTGCTTGGCTATATTGGAT
TTGTTGGACGGTCGCCTTGGGGACTGAATTTTTAGGTGCTGCCATGCTGAT
GCAGCGCTGGTTCCCAAATGTGCCGGCTTGGGCATTTGCTTCCTTTTTTGCC
CTTGTGATTTTTGGTTTAAATGCTCTTAGCGTACGCTTTTTTGCAGAAGCAG
AGTCTTTCTTCTCAAGTATTAAGGTTATTGCTATCATTATCTTTATTATCTTG
GGCTTAGGTGCTATGTTTGGTCTAGTTTCCTTTGAAGGTCAGCACAAGGCT
ATTCTCTTCACTCATCTGACTGCCAATGGTGCCTTTCCAAATGGTATCGTTG
CAGTTGTCTCAGTCATGTTGGCTGTTAACTATGCCTTCTCTGGTACTGAGTT
AATTGGTATTGCGGCTGGTGAAACGGATAATCCCAAAGAAGCTGTACCAA
GGGCTATTAAAACGACAATCGGTCGCTTGGTTGTTTTCTTTGTACTGACAA
TTGTTGTCCTAGCTTCGCTATTGCCAATGAAAGAGGCAGGCGTATCCACAG
CACCATTCGTTGATGTCTTTGACAAGATGGGAATCCCTTTTACGGCGGATA
TCATGAACTTCGTTATCTTGACAGCCATCCTGTCTGCTGGTAACTCAGGTCT
CTACGCATCAAGCCGTATGCTCTGGTCCCTTGCCAATGAAGGTATGTTGTC
AAAATCTGTTGTGAAAATCAATAAACACGGTGTCCCAATGCGTGCTCTTCT
CTTGTCAATGGCAGGAGCAGTGCTGTCGCTCTTTTCAAGTATTTACGCTGC
AGACACAGTTTATCTAGCCTTGGTTTCAATCGCGGGCTTTGCTGTTGTTGTC
GTATGGCTAGCCATTCCAGTCGCACAAATCAATTTCCGCAAGGAATTC

MENHRYEDEGKFQRKMTSRHLFMLSLGGVIGTGLFLSSGYTIAQAGPLGAVL
SYLIGAVVVYLVMLSLGELAVAMPVTGSFHTYATKFISPGTGFTVAWLYWIC
WTVALGTEFLGAAMLMQRWFPNVPAWAFASFFALVIFGLNALSVRFFAEAES
FFSSIKVIAIIIFIILGLGAMFGLVSFEGQHKAILFTHLTANGAFPNGIVAVVSVM
LAVNYAFSGTELIGIAAGETDNPKEAVPRAIKTTIGRLVVFFVLTIVVLASLLPM
KEAGVSTAPFVDVFDKMGIPFTADIMNFVILTAILSAGNSGLYASSRMLWSLA
NEGMLSKSVVKINKHGVPMRALLLSMAGAVLSLFSSIYAADTVYLALVSIAGF
AVVVVWLAIPVAQINFRKEF

Sequence description:

A] Length: 1126 bp - 393 aa (partial gene

FIG. 1 CONT'D

sequence)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-145 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-145 gene sequence. Putative ATG start codon is preceded by a typical Shine-Dalgarno sequence. Possesses a possible leader peptide sequence.

ID-190

Clone 3-94b

TCAGAAAATGCAGAGGCAGCAACGGTTGCCACAAACTTGGTTACCAAAGG
AGCTAATGTCATTATCGGACCAGCAACATCGGGTGCAGCTGCATCTTCAAC
TCCAAAAGTAAATGCAGCAGCAGTTCCAATGATTGCACCTGCTGCGACAC
AAGACAATTTAGTCTATGGTTCTGATGGAAAAACCTTAAATCAGTATTTCT
TCCGAGCTACTTTTGTCGATAATTATCAAGGAAAGCTATTGTCTCAGTATG
CTACAGACAACCTTAAAGCTAAAAAAGTTGTTCTATTTTATGATAATTCAT
CAGATTACTCAAAGGGGGTAGCAAAATCATTTAAGGAAAGTTATAGTGGA
AAAATTGTTGATAGTATGACATTCTCCGCTGGTGATACTGATTTCCAAGCG
TCATTGACTAAGTTGAAAGGGAAAGAATATGATGCTATTGTGATGCCAGG
TTACTATACCGAGACAGGATTAATAGTTAAGCAAGCGCGTGATTTAGGTAT
CTCTAAACCGGTTCTTGGGCCTGATGGTTTTGATAGTCCGAAATTTGTGCA
ATCGGCAACACCTGTAGGAGCTTCAAACGTTTATTATTTGACAGGTTTCAC
TACACAAGGATCAACCAAAGCTAAAGCT

SENAEAATVATNLVTKGANVIIGPATSGAAASSTPKVNAAAVPMIAPAATQD
NLVYGSDGKTLNQYFFRATFVDNYQGKLLSQYATDNLKAKKVVLFYDNSSD
YSKGVAKSFKESYSGKIVDSMTFSAGDTDFQASLTKLKGKEYDAIVMPGYYT
ETGLIVKQARDLGISKPVLGPDGFDSPKFVQSATPVGASNVYYLTGFTTQGST
KAKA

Sequence description

A] Length: 637 bp - 231 aa (partial sequence)

FIG. 1 CONT'D

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-149 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-149 gene sequence. N- and C-termini have yet to be determined

ID-191

Clone 2-c94b (ID-153b)

TTGGGACTTAAAGACCATGCTTTAGTCTATCCATTTTCATTATCTGGGGGG
CAAAAGCAACGTGTCGCACTAGCTCGTGCGATGATGATTGATCCACAGATT
ATTGGTTATGATGAGCCAACTAGCGCTCTTGATCCAGAGTTGCGTCAAGAA
GTAGAAAAACTAATTTTACAAAATAGAGAAACAGGTATGACACAAATTGT
AGTAACACATGATCTTCAATTTGCTGAAAGTATATCTGATACGATTCTCAA
AATTAATCCTAAGTAG

MGLKDHALVYPFSLSGGQKQRVALARAMMIDPQIIGYDEPTSALDPELRQEV
EKLILQNRETGMTQIVVTHDLQFAESISDTILKINPK*

Sequence description

A] Length: 270 bp - 90 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-153 gene which was identified by LEEP, during cloning and sequence analysis of the ID-153 gene sequence.
N-terminus has yet to be determined

ID-192

Clone 2-c1b (ID-155b)

ATGACTAATATCTCAGATGTTCCAAAAGCTATTAGAACACAGGCACAGTAT
GTTCTCTTGGGAATGAGAGTTATGGATCAGTCGGTATTACCGAAAACATAT
AATTCAAAAGAACCTTATTTGAAACCAGATATGATTTATATTCATGATAGA

FIG. 1CONT'D

AGACAAGAGACAATGCTTAAAATCACTCAAGAAATAGAAATGGAGCATTG
A

MTNISDVPKAIRTQAQYVLLGMRVMDQSVLPKTYNSKEPYLKPDMIYIHDRR
QETMLKITQEIEMEH*

Sequence description

A] Length: 204 bp - 68 aa (partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-155 gene which was identified by LEEP, during cloning and sequence analysis of the ID-155 gene sequence.
ATG start codon is preceded by a potential typical Shine-Dalgarno sequence. Has a
typical leader peptide. N-terminus has yet to be
verified

ID-193

Clone 2-54altb (ID-172b)

AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCTTGGGGAATATAAATT
TGGATTTCATGACGATGTAAAGCCAATTTATTCTACGGGAAAAGGTCTAAA
TGAGGCTGTTATTCGTGAGTTATCTGCAGCTAAGGGTGAACCTGAGTGGAT
GTTGGACTTTCGTCTAAAATCCTTGGAAACGTTTAATAAAATGCCGATGCA
GACCTGGGGAGCAGATTTATCAGATATTGATTTTGATGATATTATTTATTA
TCAAAAAGCATCTGATAAACCTGCGCGTGATTGGGATGATGTTCCAGAAA
AAATCAAAGAAACTTTTGAAAGAATTGGGATTCCAGAAGCTGAAAGAGCC
TATCTTGCAGGAGCATCAGCACAATATGAATCAGAAGTAGTTTATCACAAT
ATGAAAGAAGAATATGATAAGCTGGGTATTGTTTTTACGGATACTGACTCT
GCACTTAAAGAGTACCCAGAGCTATTCAAAAAATATTTTGCTAAACTTGTC
CCTCCAACAGATAATAAATTAGCTGCTCTGAACTCTGCTGTATGGTCAGGT
GGAACATTTATTTATGTTCCTAAAGGTGTTAAGGTGGATATTCCACTTCAA
ACTTACTTCCGTATTAATAATGAAAATACTGGACAATTTGAACGTACTCTC
ATTATTGTTGATGAGGGAGCAAGTGTTCACTATGTTGAAGGTTGTACCGCC
CCAACTTATTCTTCAAATAGTTTACATGCAGCTATAGTTGAAATTTTTGCAC
TTGATGGAGCTTATATGCGCTATACGACTATTCAAAATTGGTCCGATAATG
TCTATAATTTAGTGACAAAACGTGCTACCGCTAAAAAAGATGCAACAGTT
GAGTGGATAGATGGAAATCTAGGAGCTAAAACAACAATGAAATACCCATC

FIG. 1CONT'D

GGTTTACCTTGATGGTGAAGGAGCACGTGGCACGATGTTGTCTATTGCTTT
TGCAAACAAAGGACAACACCAAGATACGGGTGCAAAGATGATTCATAATG
CCCCCCATACTAGTTCATCCATTGTCTCTAAATCAATTGCTAAGGGTGGGG
GAAAAGTTGATTATCGAGGTCAAGTGACATTTAATAAAGATTCCAAAAAA
TCAGTGTCACATATAGAATGTGACACCATATTGATGGATGATATTTCAAAA
TCAGATACCATACCGTTTAATGAGATTCATAATTCACAGGTTGCTTTAGAG
CATGAAGCAAAGGTGTCTAAGATTTCTGAAGAGCAACTGTACTACTTGATG
AGTCGAGGTTTATCTGAAGCTGAAGCAACAGAAATGATTGTTATGGGGTTT
GTTGAGCCCTTTACGAAAGAATTACCAATGGAATATGCGGTAGAGTTAAA
TCGTTTAATTTCCTATGAAATGGAAGGTTCAGTTGGTTAA

MHACRSTLEDLGEYKFGFHDDVKPIYSTGKGLNEAVIRELSAAKGEPEWMLD
FRLKSLETFNKMPMQTWGADLSDIDFDDIIYYQKASDKPARDWDDVPEKIKE
TFERIGIPEAERAYLAGASAQYESEVVYHNMKEEYDKLGIVFTDTDSALKEYP
ELFKKYFAKLVPPTDNKLAALNSAVWSGGTFIYVPKGVKVDIPLQTYFRINNE
NTGQFERTLIIVDEGASVHYVEGCTAPTYSSNSLHAAIVEIFALDGAYMRYTTI
QNWSDNVYNLVTKRATAKKDATVEWIDGNLGAKTTMKYPSVYLDGEGARG
TMLSIAFANKGQHQDTGAKMIHNAPHTSSSIVSKSIAKGGGKVDYRGQVTFN
KDSKKSVSHIECDTILMDDISKSDTIPFNEIHNSQVALEHEAKVSKISEEQLYYL
MSRGLSEAEATEMIVMGFVEPFTKELPMEYAVELNRLISYEMEGSVG*

Sequence description:

A] Length: 1411 bp - 469 aa (Possible full-length gene)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-72 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-72 gene sequence. No obvious Shine Dalgarno sequence upstream of TTG start codon insufficient sequence data). N terminus needs verification.

ID-194

Clone 3-1b (ID-81b)

ATGATAGAATTCTTTTCTAATATCAGAACAGAGATTCCGCAGATGCCTTTA
CTTATCCATAGTTTGATTTTATCTGTCTTACCTTTTCTGATGTGGCTGACTTT
GGTTAATAGAGATAAGCCTTTGTATAAAACTATTTGGAGTATCCTTTTAGG
ACTTCAGTTAATTACGATTTATACTTGGTTTTTCTGGGCAAAATTGCCTTTA

FIG. 1CONT'D

TCTGAAAGTCTTCCCCTTTACCATTGTCGAATAGGCATGTTTGTCGGTCTCT
TA

MIEFFSNIRTEIPQMPLLIHSLILSVLPFLMWLTLVNRDKPLYKTIWSILLGLQLI
TIYTWFFWAKLPLSESLPLYHCRIGMFVGLL

Sequence description
A) Length: 261 bp - 87 aa (partial gene sequence)
B) This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-81 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-81 gene sequence. Sequence Characteristics: Possesses a potential leader peptide sequenceOrf is preceded by a potential Shine-Dalgarno sequence.

ID-195

Clone RS-55b

AAGCTTGTGCAAAGTATTAAAGAGATAGGATTAGCTAATGCGCATTTATTA
GCTGTTGCTCCGACAGGGTCAATCAGTTATCTTTCTTCTTGTACTCCGAGCC
TTCAACCGGTTGTATCACCTGTCGAAGTACGCAAGGAAGGAGCACTGGGG
AGGGTTTATGTAGCTGCTTATAAGATTGATGCAGATAATTATGTCTACTAC
AAAAAAGGAGCTTATGAAGTGGGATCTGAGGCGATTATCAATATTGCAGC
TGCCGCTCAAAAACACATTGATCAAGCTATTTCGTTAACGCTTTTCATGAC
AGATCAAGCAACTACGCGAGATTTAAATAAAGCCTATATTCAAGCATTTA
AACAAAAATGTGCCTCTATTTATTATGTACGAGTGAGACAGGACATCCTAG
AAGGTAGCGAGAGTTATGATGATATGCTGGATGATTTCACTTCATCGGACT
TAGAAGACTGTCAATCCTGCATGATTTAA

>KLVQSIKEIGLANAHLLAVAPTGSISYLSSCTPSLQPVVSPVEVRKEGALGRV
YVAAYKIDADNYVYYKKGAYEVGSEAIINIAAAAQKHIDQAISLTLFMTDQAT
TRDLNKAYIQAFKQKCASIYYVRVRQDILEGSESYDDMLDDFTSSDLEDCQSC
MI*

Sequence description:

FIG. 1 CONT'D

A] Length 486 bp - 162 aa (Partial sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-87 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-87 gene sequence. N-terminus to be determined.

ID-196

Clone RS-59(ID-90b)

GTGAGGACATATATTACAAACTTGAATGGACATTCAATCACTAGTACAGC
ACAAATAGCTCAAAACATGGTAACAGATATAGCAGTAAGCTTAGGTTTTC
GTGAGCTGGGAATACATTCTTATCCGATTGATACTGATTCTCCTGAGGAAA
TGAGTAAGCGTTTAGATGGAATCTGTTCCGGACTTAGAAAAAATGATATTG
TCATATTTCAGACACCTACATGGAACACTACAACTTTTGATGAAAAATTAT
TTCACAAATTAAAAATATTTGGTGTAAAGATTGTTATTTTTATACATGATGT
TGTACCGCTAATGTTTGATGGAAATTTTTATTTGATGGATAGAACTATAGC
TTATTATAATGAAGCAGATGTTTAATAGCCCCTAGTCAAGCAATGGTCGAT
AAGCTT

MRTYITNLNGHSITSTAQIAQNMVTDIAVSLGFRELGIHSYPIDTDSPEEMSKRL
DGICSGLRKNDIVIFQTPTWNTTTFDEKLFHKLKIFGVKIVIFIHDVVPLMFDGN
FYLMDRTIAYYNEADVLIAPSQAMVDKL

Sequence description:

A] Length: 414 bp - 138 aa(partial gene)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-90 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-90 gene sequence. No obvious signal peptide, but a possible Shine Dalgarno sequence is present upstream of ATG start codon. C-terminus has yet to be determined.

ID-197

Clone RS-59c (ID-90c)

FIG. 1CONT'D

CATGGAAATGAAGTTGATGATGTTATTAGAAGGGCATTTGAATATAATCAC
CTTATCTTTGCTTTTGATAATACCTGTCATAACAGAGAGTTAGTATTAGATA
GCAATATCATTTCTCACACAACCTGTGAACAATTGATAAATTTAATGAAAA
ATTTATCAGGCTCCATTATGTATTTGCTAGAGCAACAAAGAGAACAAACA
AGTAATGAAACAAAAGAGCGTTATAAAGAAATATTAGGAGGGTATGGAA
ATGCCTAA

HGNEVDDVIRRAFEYNHLIFAFDNTCHNRELVLDSNIISHTTCEQLINLMKNLS
GSIMYLLEQQREQTSNETKERYKEILGGYGNA*

Sequence description:

A] Length: 261 bp - 87 aa(partial gene sequence)
B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-90 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-90 gene sequence. N-terminus has yet to be determined

ID-198

Clone RS-70b (ID-93b)

ACATTTTTATATTATGTATTTGAAGACGTAGCCACCCAGTCAAATATGACT
GGGAAGATTTTTAGTATGTCTAAAGAAGAGTTGTCATATTTACCCGTTATT
AAACTTTTTAAGAATCAAGGTGTATACAACGGCTTGATTGGTCTATTCCTC
CTTTATGGGTTATATATTTCACAGAATCAAGAAATTGTAGCTATTTTTTTAA
TCAATGTGTTGCTAGTTGCTGTTTATGGTGCTTTGACAGTTGATAAAAAAA
TCTTATTAAAACAGGGTGGTTTACCTATATTAGCTCTTTTAACATTCTTATT
TTAA

TFLYYVFEDVATQSNMTGKIFSMSKEELSYLPVIKLFKNQGVYNGLIGLFLLY
GLYISQNQEIVAIFLINVLLVAVYGALTVDKKILLKQGGLPILALLTFLF*

Sequence description:

A] Length: 312 bp - 104 aa (partial gene sequence)

FIG. 1CONT'D

B] This gene sequence was not identified using the LEEP system. It was identified upstream of the ID-93 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-93 gene sequence.
N-terminus has yet to be determined

ID-199

Clone RS-70c (ID-93c)

ATGAAATTAAGTGTCCTTGATTATGGGCTTATTGATTATGGAAAAACTGCA
AGTGATGCAATACAAGAAACGATTCTTTTATCACAAGAGGCGGAGCAACT
AGGCTATCATCAATTTTGGGTGGCTGAACATCACGGTGTTAAGGCATTCAG
TATTAGCAATCCAGAATTAATGATAATGCATTTGGCTAACCAGACTAAATC
TATCAAAATTGGCTCTGGAGGTATAATGCCTCTGCACTATAGTAGTTTTAA
ACTCGCGGAGACTCTCAAGACATTAGAGACATGTCATCCGAATCGAGTAA
GTATTGGTTTAGGAAATTCACTAGGGACAGTTAAAGTTTCAAATGCACTTC
GTAGCTTACATAAAGCACATGATTACGAAGAGGTACTGGAGGAATTGAAG
TCATGGCTTATTGATGAATCATCCAGTAAGGAACCATTAGTTCAACCGACT
CTTTCTAGCTTCCCAGACTTATATGTGTTGGGGAGTGGTCAAAAATCAGCT
TATTTAGCGGCTAAACTTGGCTTAGGCTTTACCTTCGGTGTTTTTCCTTTTA
TGGACAAAGACCCATTGACAGAAGCTAAA

MKLSVLDYGLIDYGKTASDAIQETILLSQEAEQLGYHQFWVAEHHGVKAFSIS
NPELMIMHLANQTKSIKIGSGGIMPLHYSSFKLAETLKTLETCHPNRVSIGLGN
SLGTVKVSNALRSLHKAHDYEEVLEELKSWLIDESSSKEPLVQPTLSSFPDLYV
LGSGQKSAYLAAKLGLGFTFGVFPFMDKDPLTEAK

Sequence description:

A] Length: 588 bp - 196 aa (partial)
B] This gene sequence was not identified using the LEEP system. It was identified downstream of the ID-93 gene which was identified by LEEP, during cloning and sequence analysis of the full-length ID-93 gene sequence. No obvious signal peptide, but Shine Dalgarno sequence upstream of the ATG start codon.

FIG. 1CONT'D

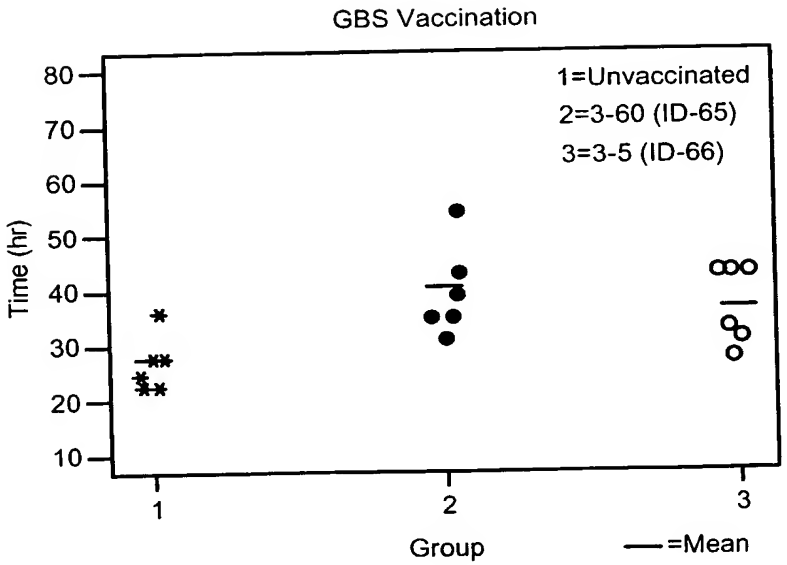

GBS Vaccination
1=Unvaccinated
2=3-60 (ID-65)
3=3-5 (ID-66)
Time (hr)
80
70
60
50
40
30
20
10
1
2
3
Group
=Mean

FIG. 2

nucS1
Bgl II Eco RV
5'-cgagatctgatatctcacaaacagataacggcgtaaatag -3'

nucS2
Bgl II Sma I
5'-gaagatcttccccgggatcacaaacagataacggcgtaaatag -3'

nucS3
Bgl II Eco RV
5'-cgagatctgatatccatcacaaacagataacggcgtaaatag -3'

nucR
Bam HI
5'-cgggatccttatggacctgaatcagcgttgtc -3'

NucSeq
5'-ggatgctttgtttcaggtgtatc -3'

$pTREP_F$
5'-catgatatcggtacctcaagctcatatcattgtccggcaatggtgtgggctttttttgttttagcggataa
caatttcacac -3'

$pTREP_R$
5'-gcggatccccgggcttaattaatgtttaaacactagtcgaagatctcgcgaattctcctgtgtgaaatt
gttatccgcta -3'

pUC_F
5'-cgccagggttttcccagtcacgac -3'

FIG. 3

V_R
5'-tcaggggggcggagcctatg -3'

V_1
5'-tcgtatgttgtgtggaattgtg -3'

V_2
5'-tccggctcgtatgttgtgtggaattg -3'

pTREP-Nuc vectors allow cloning of genomic DNA into each frame with respect to the nuclease gene

(i)

```
pTREP1-nucl (EcoRV) AAGTATCAGATCT--GATATC--TCACAAACAGATAACGGCGTAAAT Frame=+1
                    ::::::::::::    ▲    ::::::::::::::::::::::::
                    ::::::::::::         ::::::::::::::::::::::::
pTREP1-nuc2 (Sma 1) AAGTATCAGATCTTCCCCGGGA-TCACAAACAGATAACGGCGTAAAT Frame=+2
                    ::::::::::::    ▲    ::::::::::::::::::::::::
                    ::::::::::::         ::::::::::::::::::::::::
pTREP1-nuc3 (EcoRV) AAGTATCAGATCT--GATATCCATCACAAACAGATAACGGCGTAAAT Frame=+3
                                    ▲    ::::::::::::::::::::::::
                                         ::::::::::::::::::::::::
Nuclease Gene                            TCACAAACAGATAACGGCGTAAAT

Cloning site is indicated bt an arrow
```

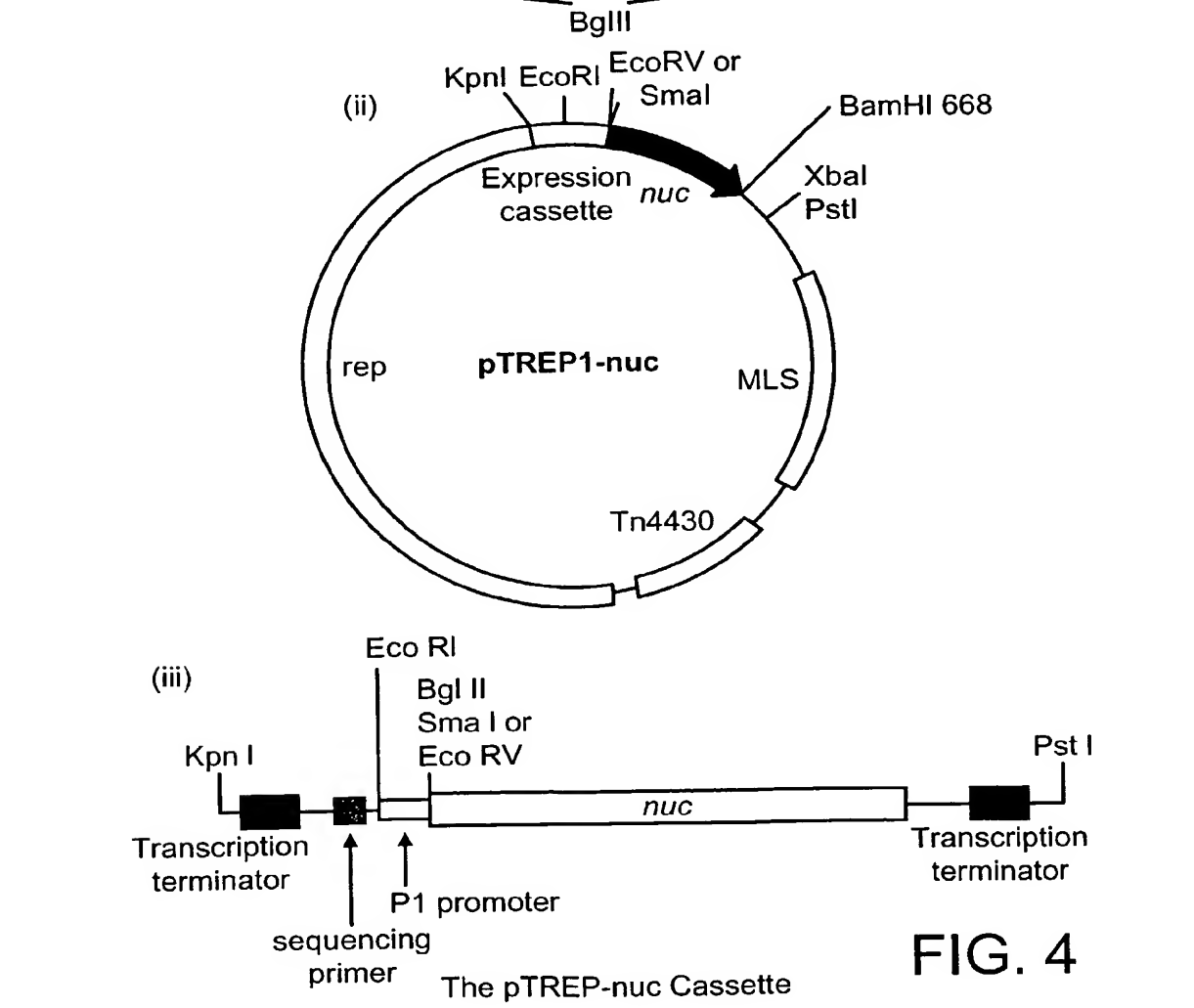


FIG. 4

FIG. 5

SDS-PAGE analysis of the purified ID-65 and ID-83 protein antigens

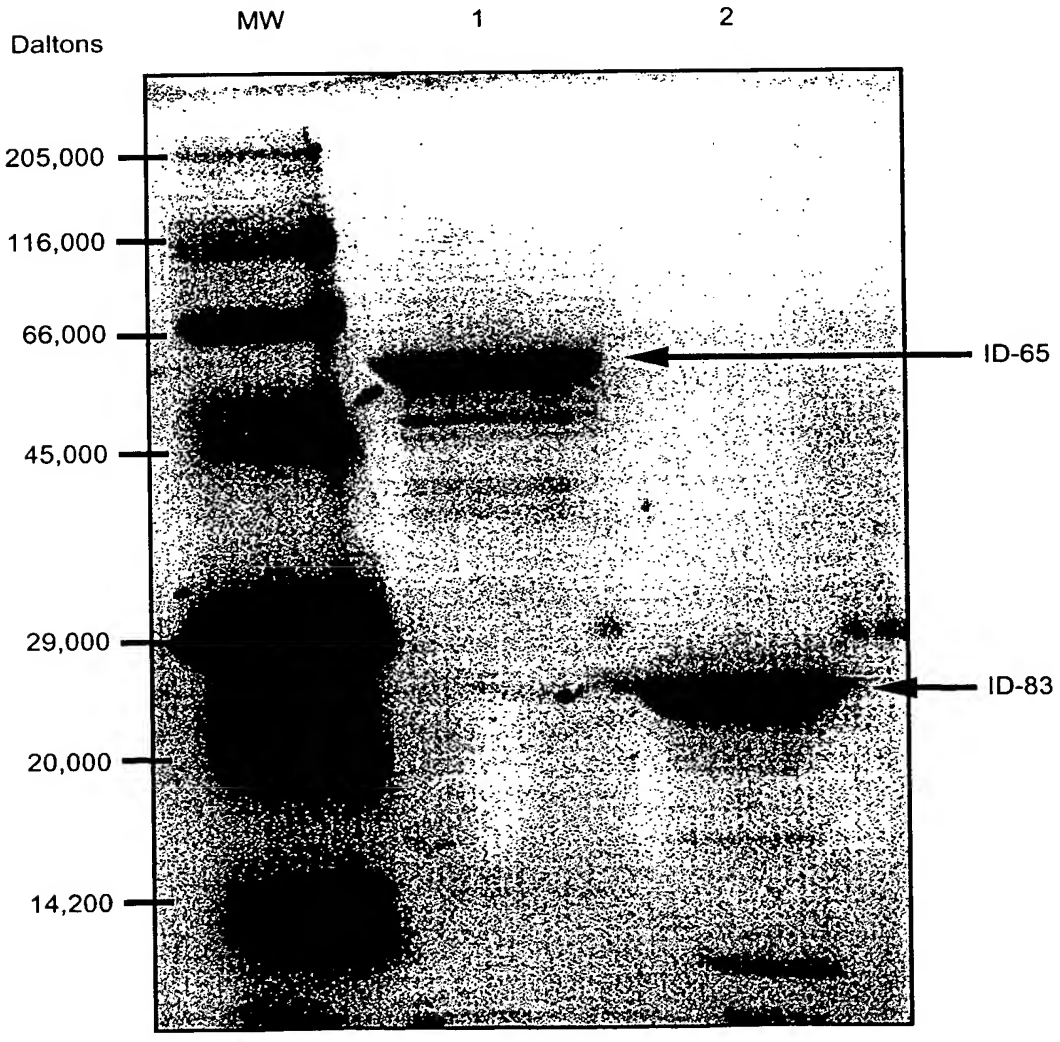


FIG. 6

SDS-PAGE analysis of the purified ID-93 antigen

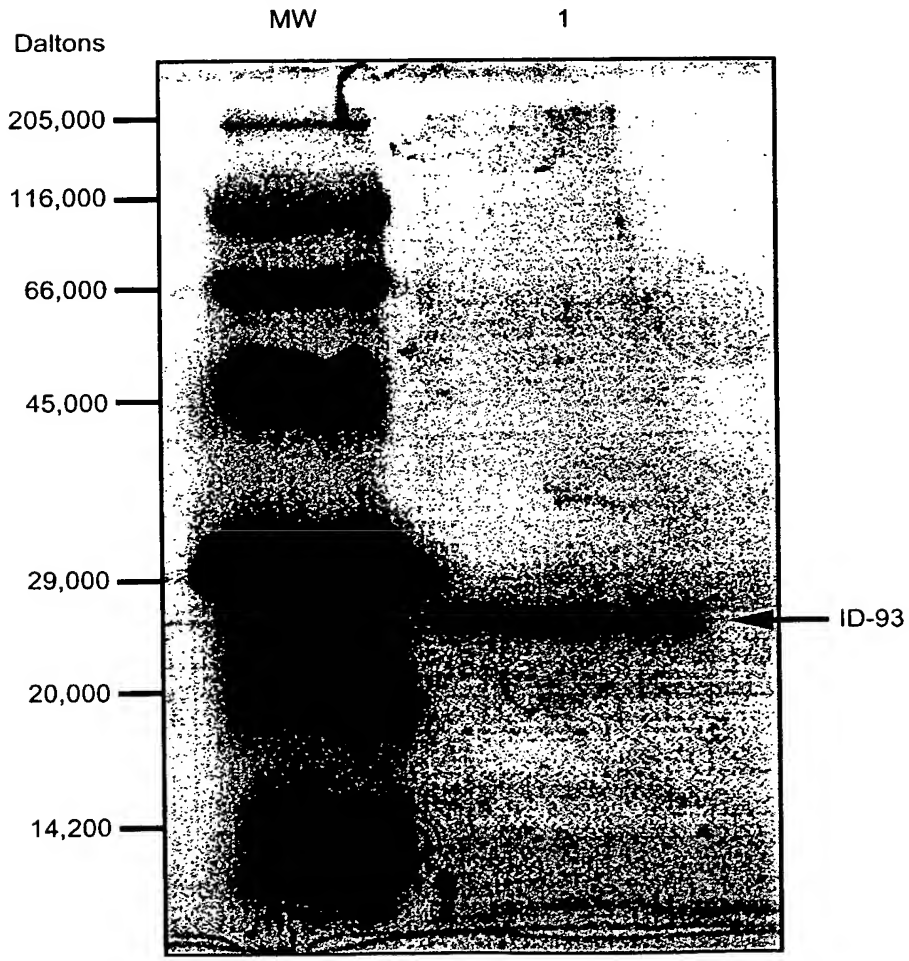


FIG. 7

SDS-PAGE analysis of the purified ID-89 and ID-96 protein antigens

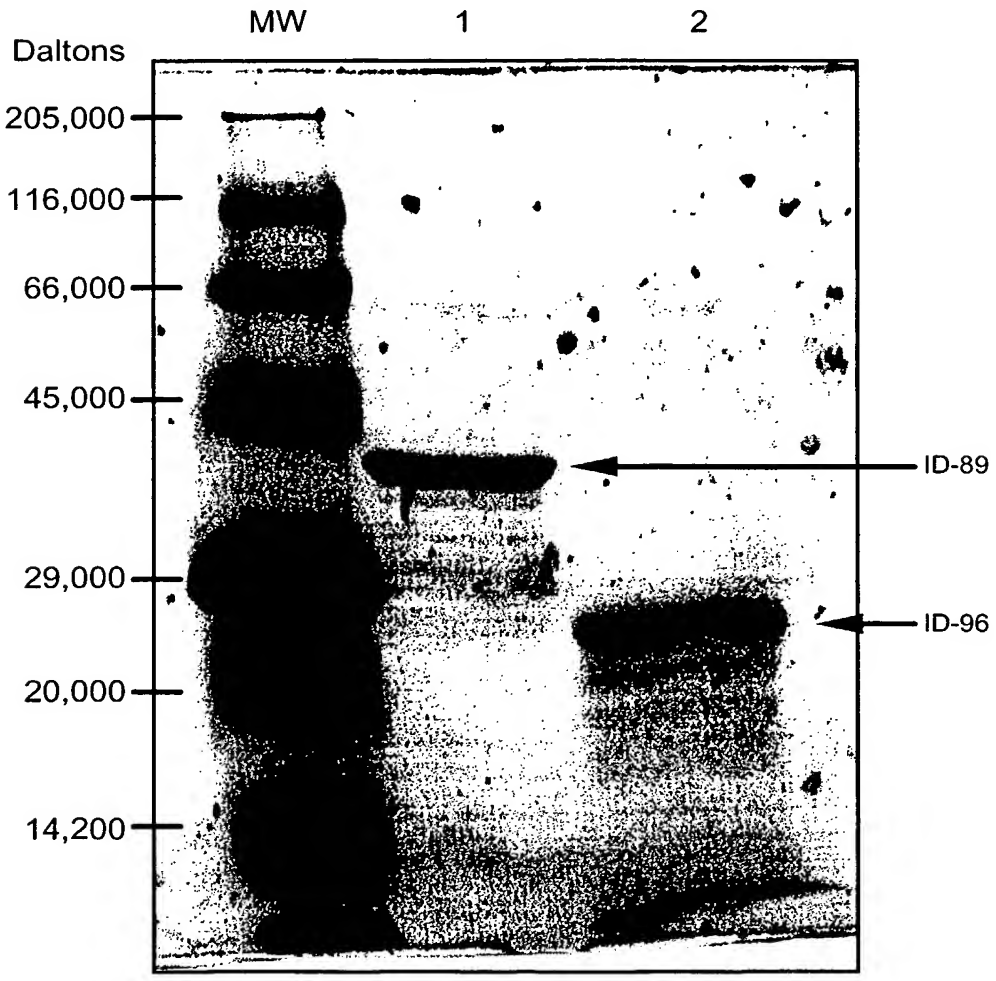


FIG. 8

IgG Titres against the ID-65 and ID-83 proteins

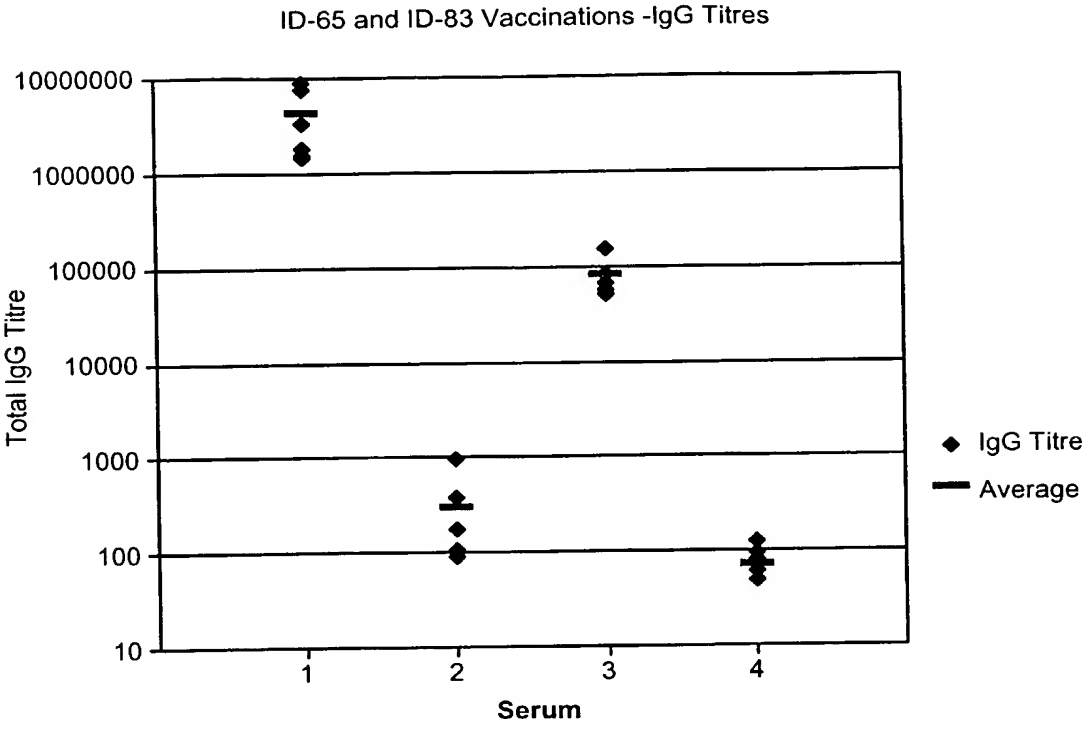


FIG. 9

Survival data

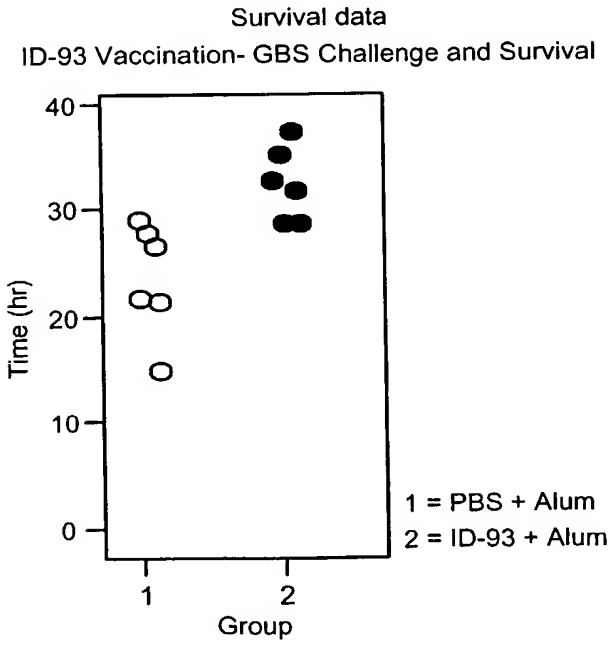


FIG. 10

IgG Titres against the ID-93 protein

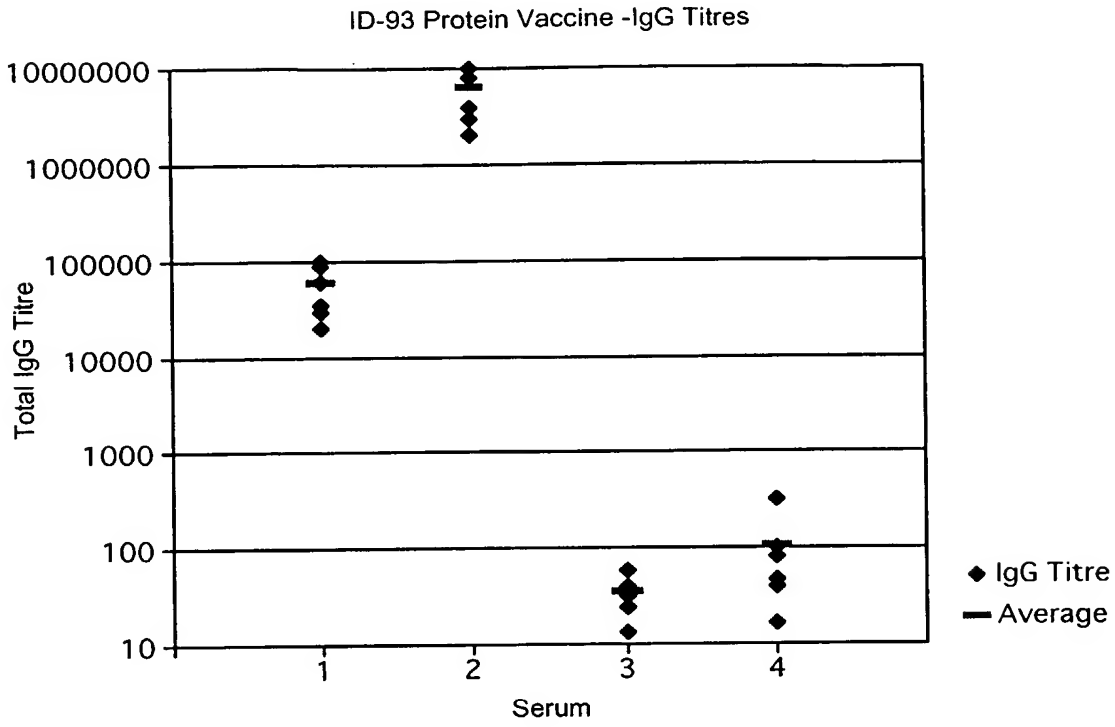


FIG. 11

IgG Titres against the ID-89 and ID-96 proteins

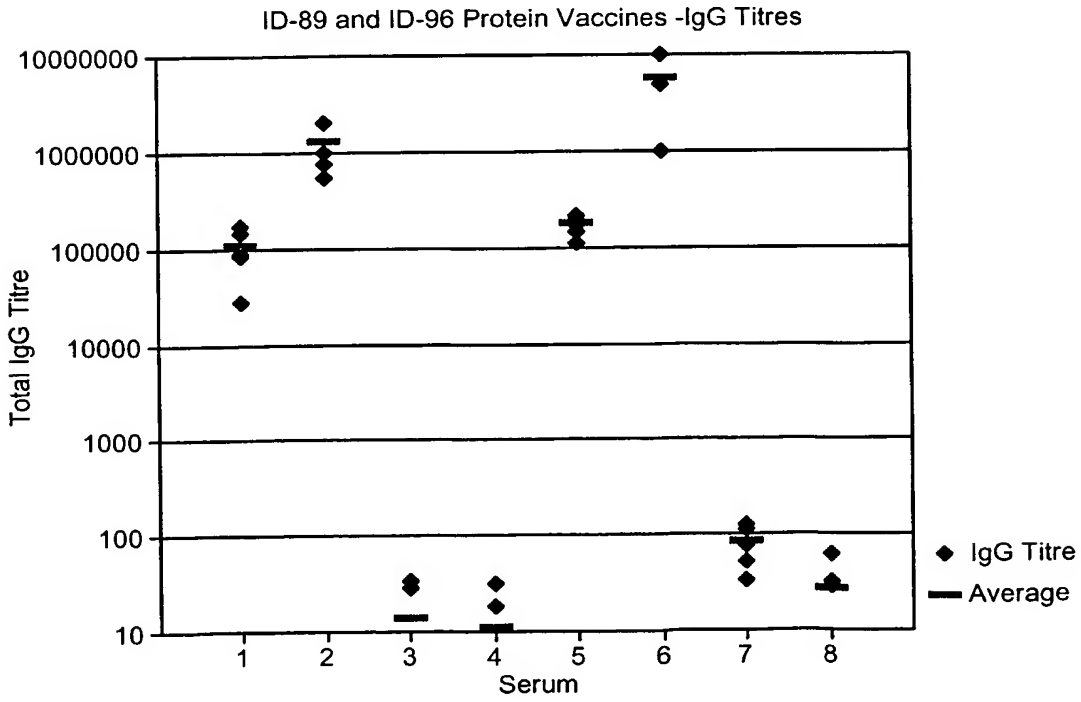


FIG. 12

Southern blot analysis - *rib*

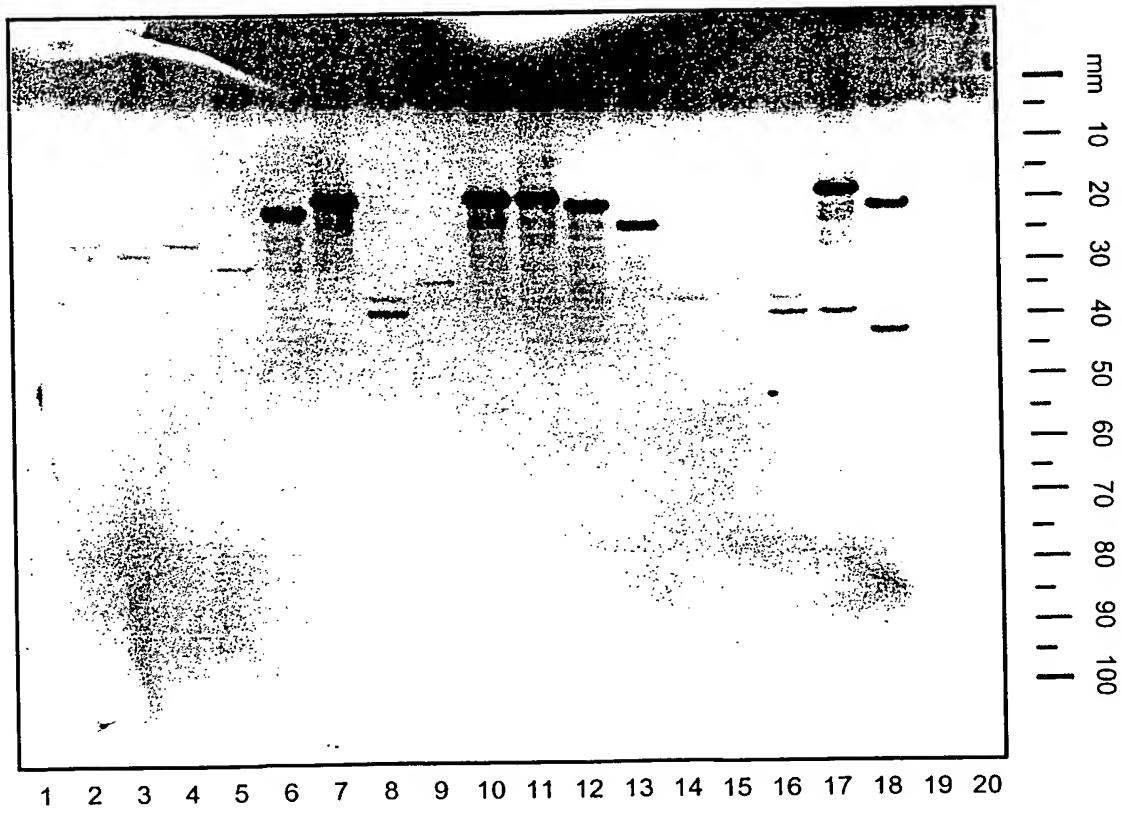


FIG. 13

Southern blot analysis - ID-65

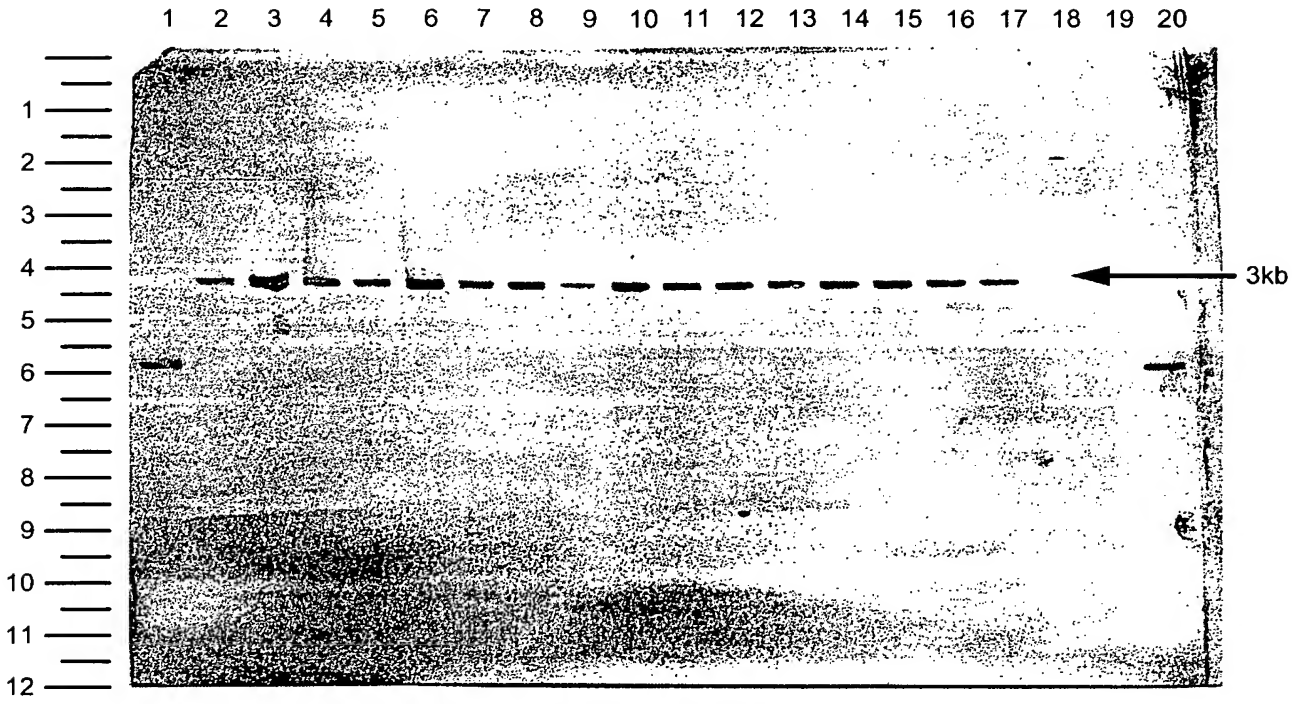


FIG. 14

Southern blot analysis - ID-89

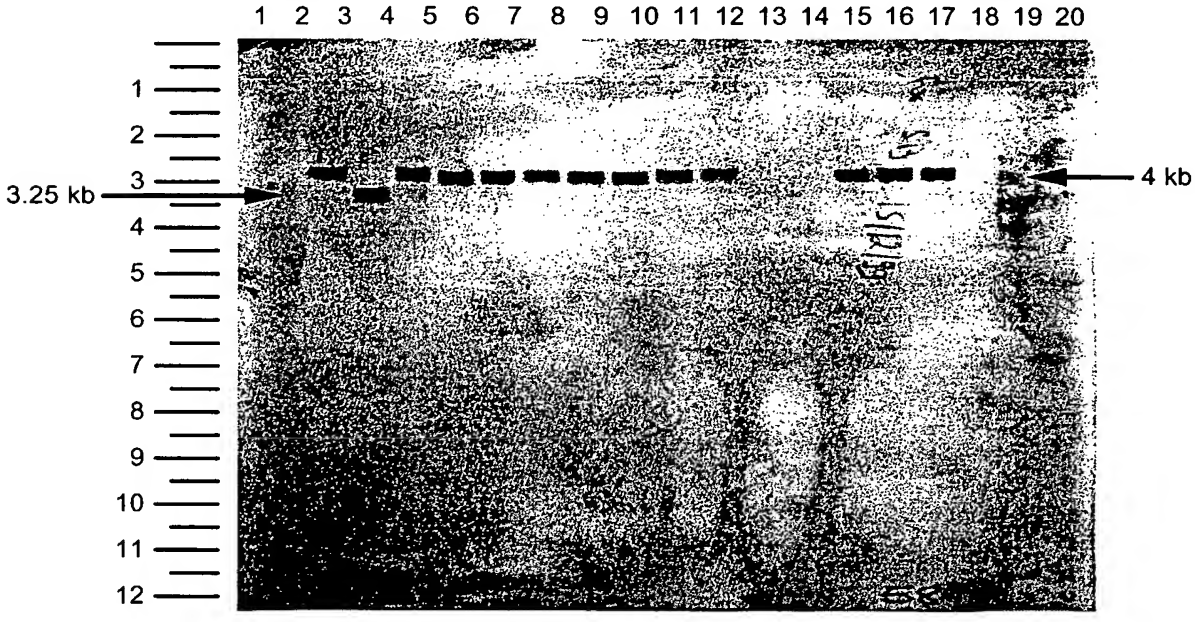


FIG. 15

Southern blot analysis - ID-93

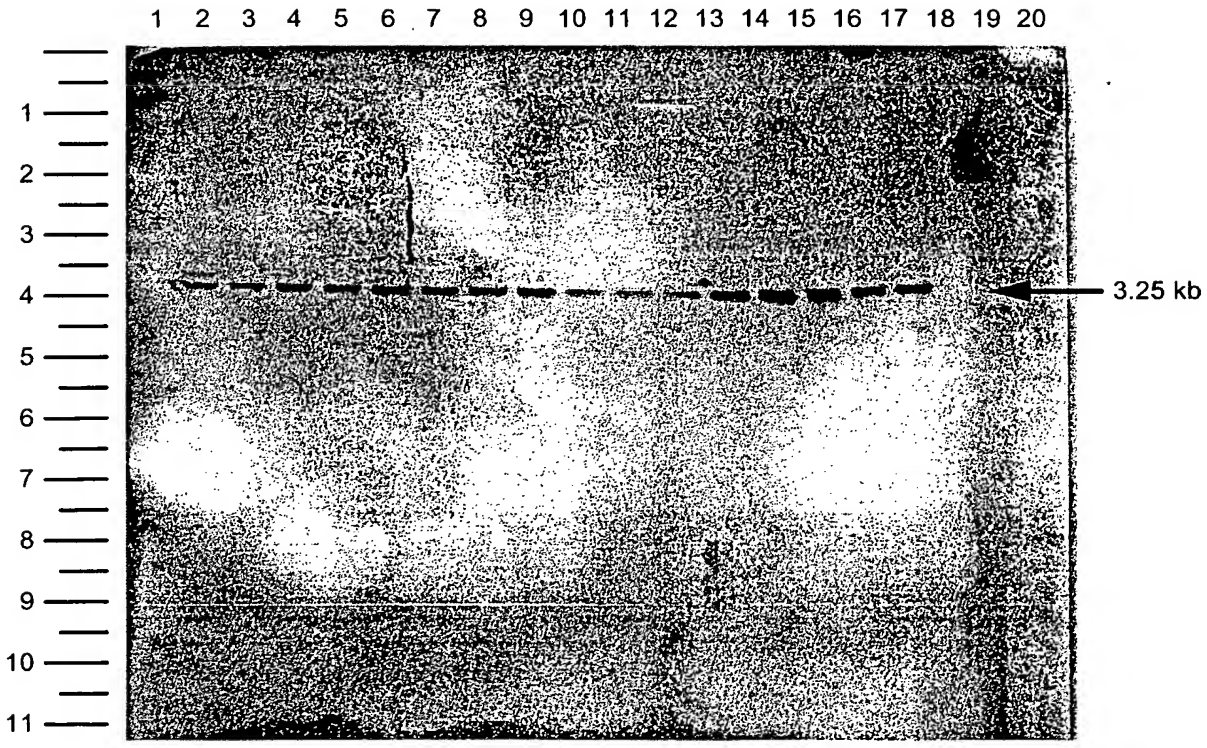
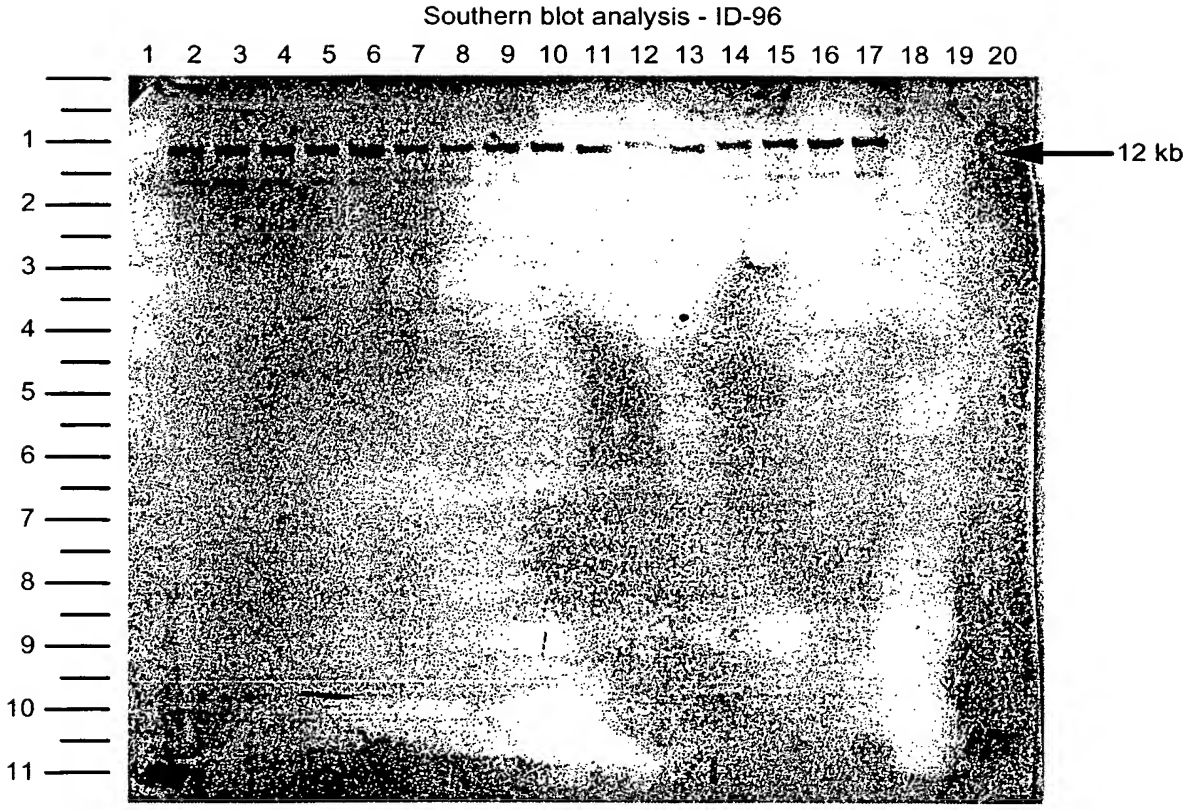


FIG. 16


Southern blot analysis - ID-96
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
1
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12 kb

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/32882 A3

(54) Title: NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

(57) Abstract: Novel protein antigens from Group B Streptococcus are described, together with the nucleic acid sequences encoding them. The use of vaccines and screening methods is also described.

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 00/03437

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/31 C12Q1/68 C12N1/21 C07K14/315 C07K16/12
A61K39/09 A61K48/00 G01N33/53 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 08553 A (UNIV CALIFORNIA) 6 March 1997 (1997-03-06) Seq Id No: 4 page 5, line 25 - line 30 claim 2 --- -/--	1-16, 21-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2001	12. 06. 2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	van Klompenburg, W

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/GB 00/03437

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SANCHEZ-BEATO: "Molecular characterization of PcpA: a novel choline-binding protein of Streptococcus pneumoniae" FEMS MICROBIOL LETT, vol. 164, no. 1, 1 July 1998 (1998-07-01), pages 207-214, XP000992682 page 213, left-hand column; figures 1,2 -& DATABASE EMBL 'Online! ebi; ACC. NO.: z82001, 30 March 1997 (1997-03-30) SANCHEZ-BEATO ET AL.: "S. pneumoniae pcpA gene and open reading frame" XP002168347 abstract	1-16, 21-23
A	MICHEL J L ET AL: "Cloned alpha and beta C-protein antigens of group B Streptococci elicit protective immunity" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 59, no. 6, June 1991 (1991-06), pages 2023-2028, XP002107260 ISSN: 0019-9567 figures 1,2,5	1-24
A	WO 99 16882 A (MEDIMMUNE INC) 8 April 1999 (1999-04-08) claims 1-12,17; figures 1-8	1-24
A	LARSSON C ET AL: "Experimental vaccination against group B Streptococcus, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alfa" INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, vol. 63, no. 9, September 1996 (1996-09), pages 3518-3523, XP002109333 ISSN: 0019-9567 figures 1-3; tables 1,2	1-24
A	US 5 928 900 A (MASURE H ROBERT ET AL) 27 July 1999 (1999-07-27) figures 1B,2-17,19,22; examples 1-4	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 00/03437

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-24 all partially

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Invention 1, claims 1-24 all partially

A Streptococcus agalactiae protein or polypeptide having a sequence as depicted in SeqIdNo.2; a homologue or derivative of said protein or polypeptide; an antigenic and/or immunogenic fragment of said protein or polypeptide; a nucleic acid molecule comprising or consisting of SeqIdNo.1, a nucleic acid molecule complementary to said sequence, a nucleic acid molecule encoding for the same or a homologue, derivative or fragment of said protein or polypeptide; use of said protein or polypeptide as an immunogen and/or an antigen; an immunogenic composition and/or antigenic composition comprising said protein or polypeptide; an antibody to said protein or polypeptide; a method of detection/diagnosis of S.pneumoniae comprising using said protein or polypeptide, said antibody, or said nucleic acid molecule; a kit for the detection of S. galactiae comprising said protein, polypeptide, antibody or nucleic acid; a method of determining whether said protein or polypeptide represents a potential antimicrobial target which comprises inactivating said protein or polypeptide and determining whether S. agalactiae is still viable.

2. Claims: Inventions 2-122, claims 1-24 all partially

Idem as subject 1 but limited to each of the polynucleotide and polypeptide sequences as in SeqIdNo:3-244, wherein invention 2 is limited to SeqIdNo:3 and SeqIdNo:4, invention 3 is limited to SeqIdNo:5 and SeqIdNo:6, ..., invention 122 is limited to SeqIdNo:243 and 244,

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int[illegible]ional Application No
PCT/GB 00/03437

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9708553 A	06-03-1997	AU 6913396 A	19-03-1997
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		EP 1037997 A	27-09-2000
US 5928900 A	27-07-1999	AU 709405 B	26-08-1999
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